FIG. 1A

pET22b(+) forward primer:
5'-CGGGATCCT TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:3)
pET22b(+) reverse primer:
5'-CCCAAGCTT TGT TCT TCT CAT ACA GAC-3' (SEQ ID NO:4)
pPICZαA forward primer:
5'-TTCGGAATTC TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:15)
pPICZαA reverse primer:
5'-TGCTCTAGAGG TGT TCT TCT CAT ACA GAC TTG GCA-3' (SEQ ID NO:16)

```
   5    10    15    20    25    30    35    40    45
tct gtt gat cac ggc ttc ctt gtg acc agg cat agt caa aca ata
  50    55    60    65    70    75    80    85    90
gat gac cca cag tgt cct tct ggg acc aaa att ctt tac cac ggg
  95   100   105   110   115   120   125   130   135
tac tct ttg ctc tac gtg caa ggc aat gaa cgg gcc cat ggc cag
 140   145   150   155   160   165   170   175   180
gac ttg ggc acg gcc ggc agc tgc ctg cgc aag ttc agc aca atg
 185   190   195   200   205   210   215   220   225
ccc ttc ctg ttc tgc aat att aac aac gtg tgc aac ttt gca tca
 230   235   240   245   250   255   260   265   270
cga aat gac tac tcg tac tgg ctg tcc acc cct gag ccc atg ccc
 275   280   285   290   295   300   305   310   315
atg tca atg gca ccc atc acg ggg gaa aac ata aga cca ttt att
 320   325   330   335   340   345   350   355   360
agt agg tgt gct gtg tgt gag gcg cct gcc atg gtg atg gcc gtg
 365   370   375   380   385   390   395   400   405
cac agc cag acc att cag atc cca ccg tgc ccc agc ggg tgg tcc
 410   415   420   425   430   435   440   445   450
tcg ctg tgg atc ggc tac tct ttt gtg atg cac acc agc gct ggt
 455   460   465   470   475   480   485   490   495
gca gaa ggc tct ggc caa gcc ctg gcg tcc ccc ggc tcc tgc ctg
 500   505   510   515   520   525   530   535   540
gag gag ttt aga agt gcg cca ttc atc gag tgt cac ggc cgt ggg
 545   550   555   560   565   570   575   580   585
acc tgc aat tac tac gca aac gct tac agc ttt tgg ctc gcc acc
 590   595   600   605   610   615   620   625   630
ata gag agg agc gag atg ttc aag aag cct acg ccg tcc acc ttg
 635   640   645   650   655   660   665   670   675
aag gca ggg gag ctg cgc acg cac gtc agc cgc tgc caa gtg tgt
 680   685   690
atg aga aga aca taa    (SEQ ID NO:1)
```

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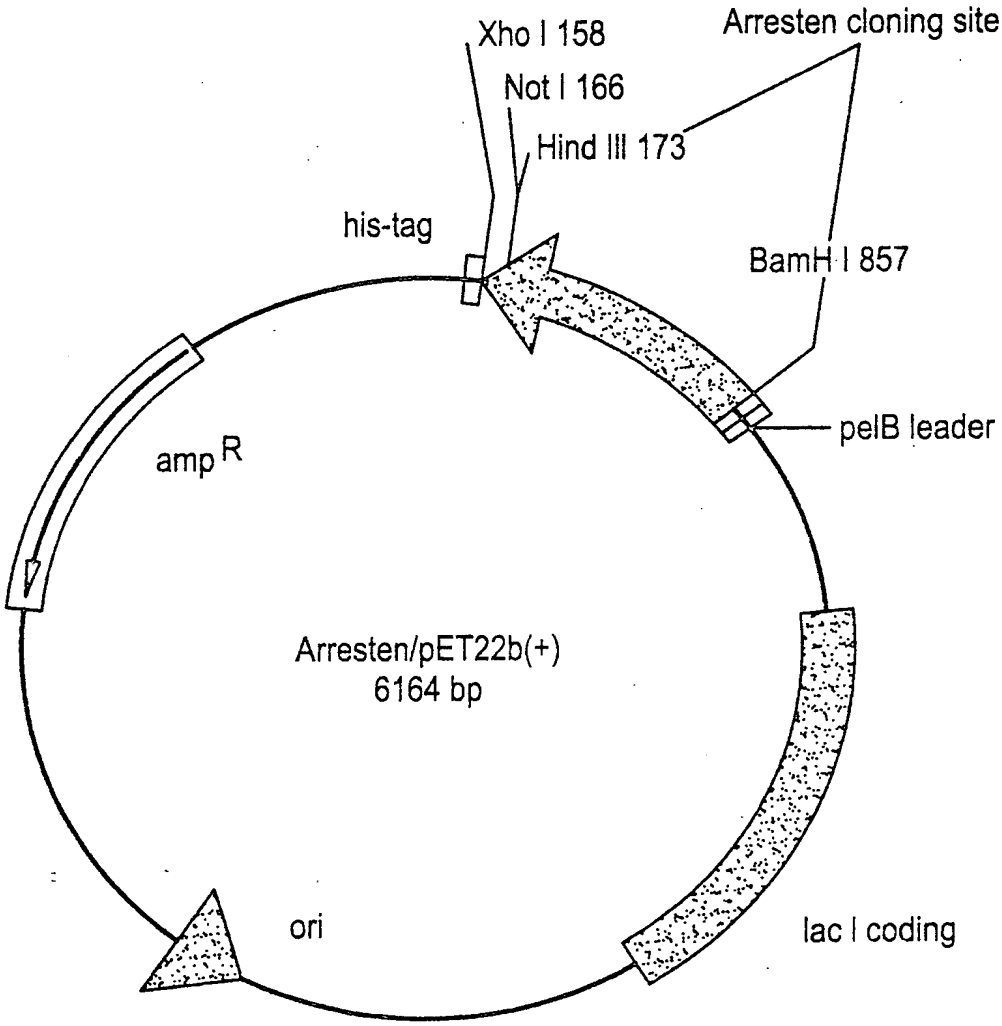
FIG. 1B

```
     5     10    15     20     25    30     35     40    45
SVD HGF LVT RHS QTI DDP QCP SGT KIL YHG YSL LYV QGN ERA HGQ

    50     55    60     65     70    75     80     85    90
DLG TAG SCL RKF STM PFL FCN INN VCN FAS RND YSY WLS TPE PMP

    95    100   105    110    115   120    125    130   135
MSM API TGE NIR PFI SRC AVC EAP AMV MAV HSQ TIQ IPP CPS GWS

   140    145   150    155    160   165    170    175   180
SLW IGY SFV MHT SAG AEG SGQ ALA SPG SCL EEF RSA PFI ECH GRG

   185    190   195    200    205   210    215    220   225
TCN YYA NAY SFW LAT IER SEM FKK PTP STL KAG ELR THV SRC QVC

  229
MRR T
```

(SEQ ID NO:2)

FIG. 2



Forward primer: 5'-cgggatccttctgttgatcacggcttc-3'

Reverse primer: 5'-cccaagctttgttcttctcatacagac-3'

FIG. 3A

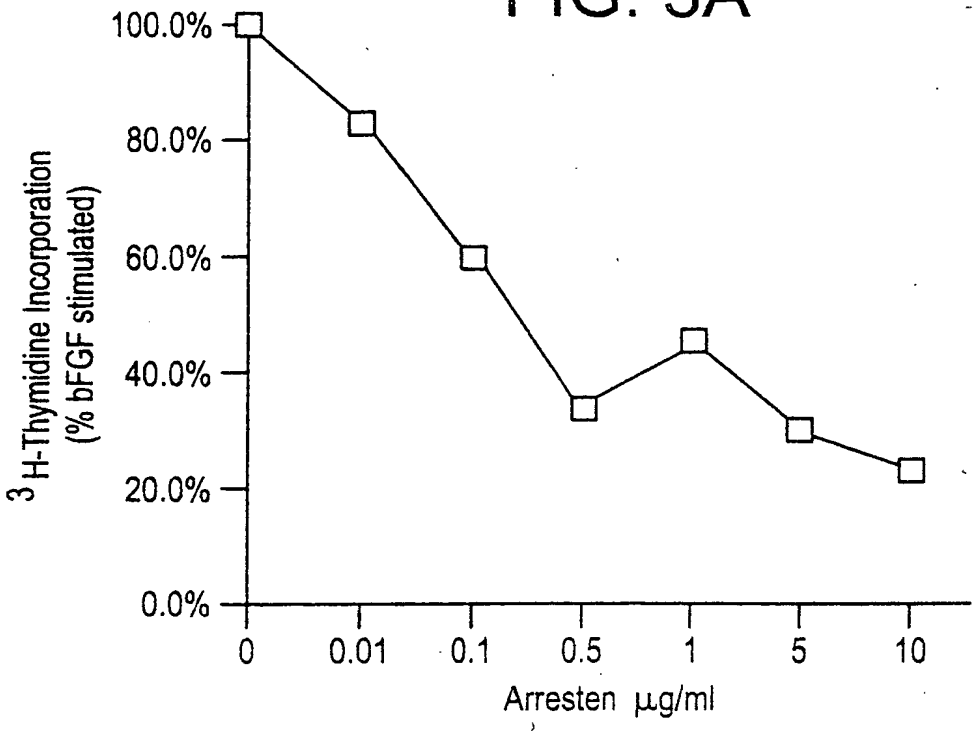

100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
^{3}H-Thymidine Incorporation
(% bFGF stimulated)
0
0.01
0.1
0.5
1
5
10
Arresten µg/ml

FIG. 3B

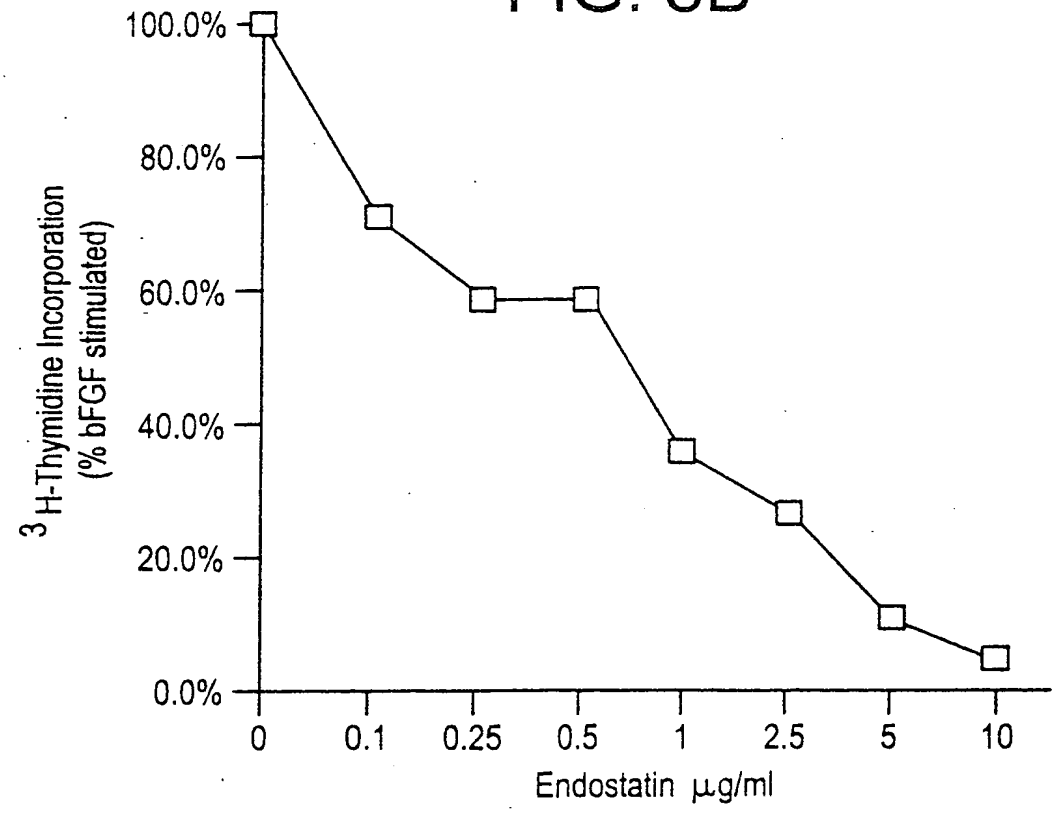

100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
^{3}H-Thymidine Incorporation
(% bFGF stimulated)
0
0.1
0.25
0.5
1
2.5
5
10
Endostatin µg/ml

FIG. 4A

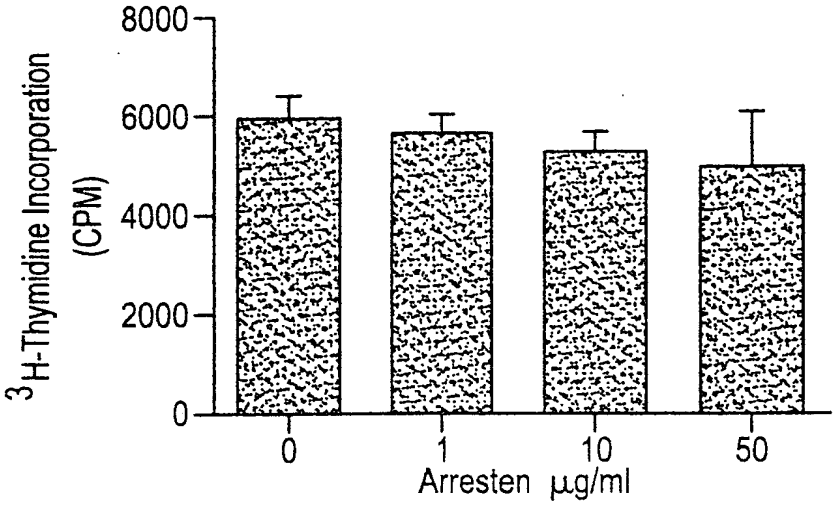

8000
6000
4000
2000
0
^{3}H-Thymidine Incorporation
(CPM)
0
1
10
50
Arresten µg/ml

FIG. 4B

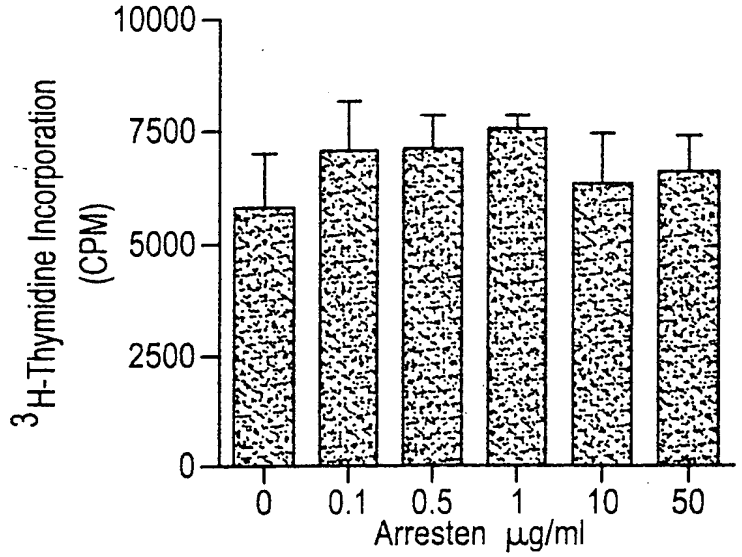

10000
7500
5000
2500
0
^{3}H-Thymidine Incorporation
(CPM)
0
0.1
0.5
1
10
50
Arresten µg/ml

FIG. 4C

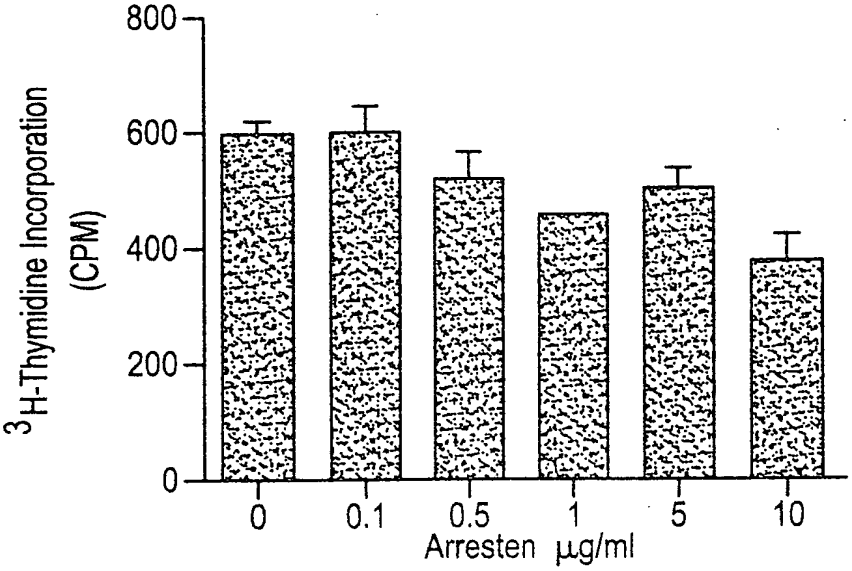

800
600
400
200
0
^{3}H-Thymidine Incorporation (CPM)
0
0.1
0.5
1
5
10
Arresten µg/ml

FIG. 4D

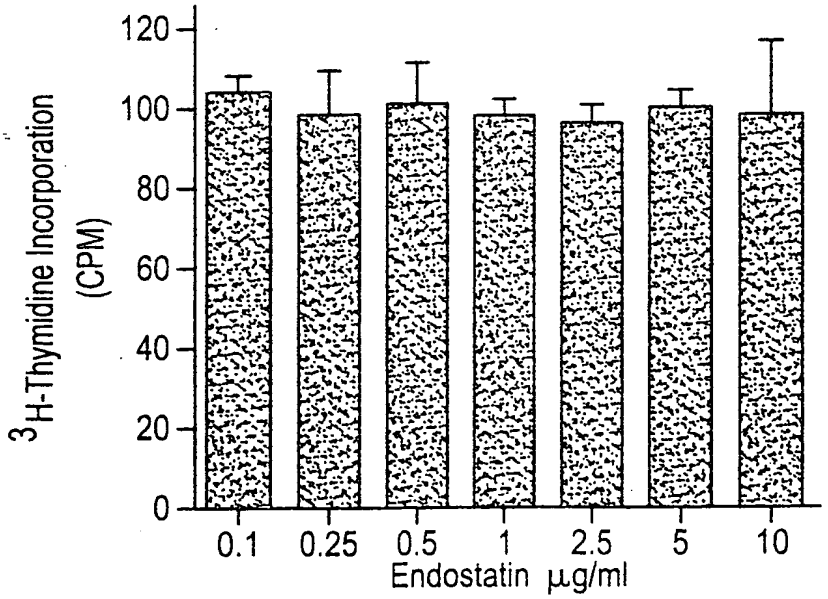

120
100
80
60
40
20
0
^{3}H-Thymidine Incorporation (CPM)
0.1
0.25
0.5
1
2.5
5
10
Endostatin µg/ml

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FIG. 5A

Control

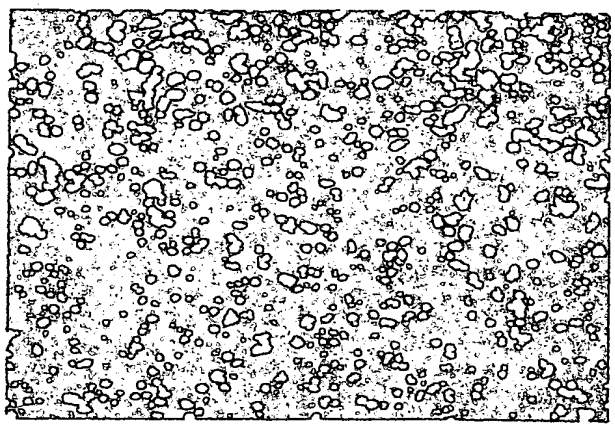


FIG. 5B

Arresten 2 μg/ml

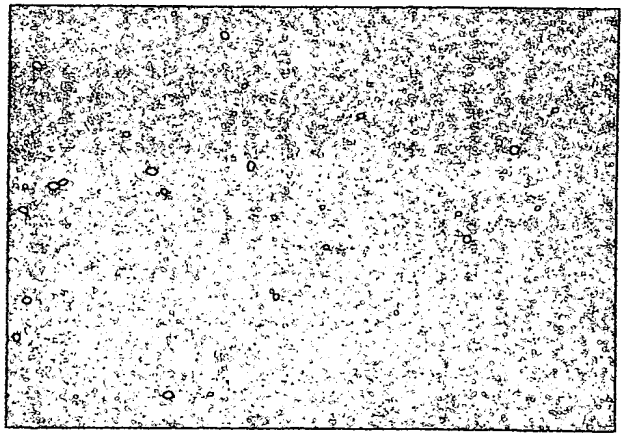


FIG. 5C

Endostatin 20 μg/ml

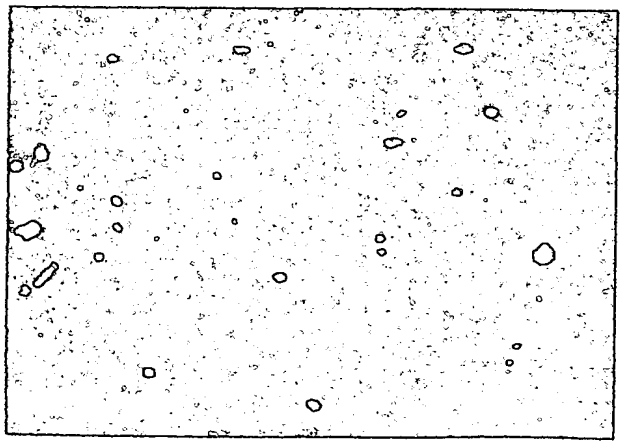


FIG. 6

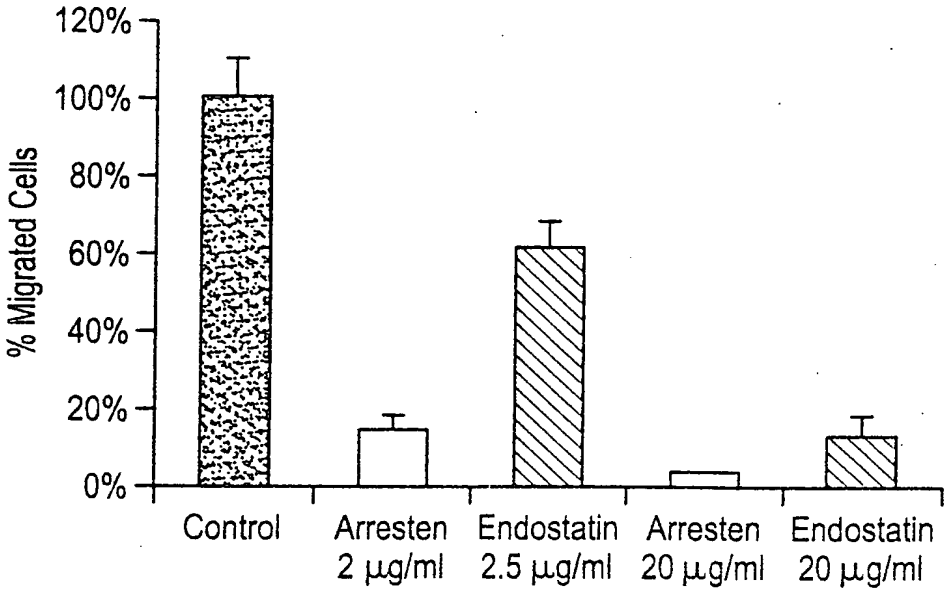

120%
100%
80%
60%
40%
20%
0%
% Migrated Cells
Control
Arresten 2 µg/ml
Endostatin 2.5 µg/ml
Arresten 20 µg/ml
Endostatin 20 µg/ml

FIG. 7

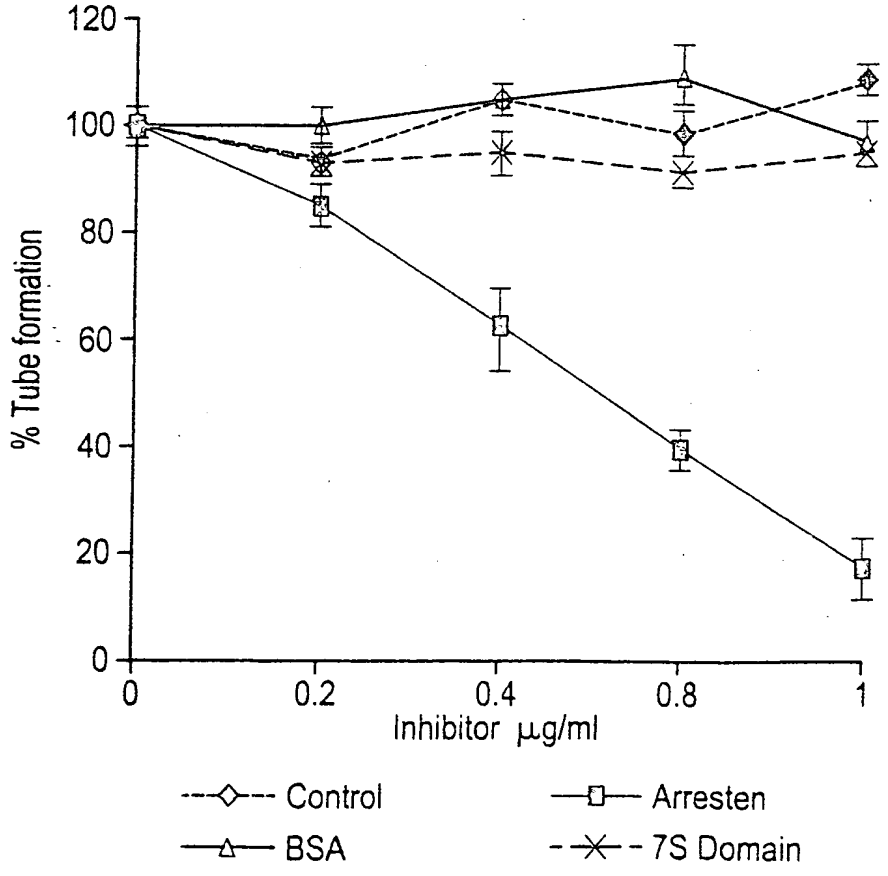

120
100
80
60
40
20
0
% Tube formation
0
0.2
0.4
0.8
1
Inhibitor µg/ml
Control
Arresten
BSA
7S Domain

FIG. 8A

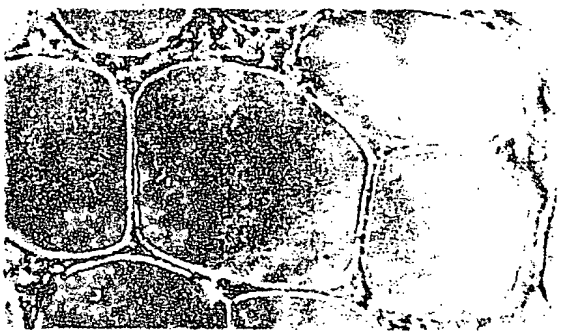


FIG. 8B

FIG. 9A

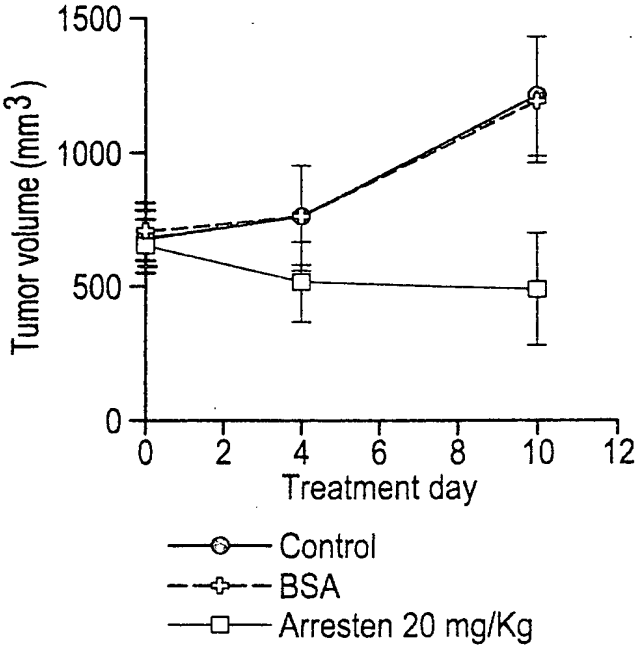


FIG. 9B

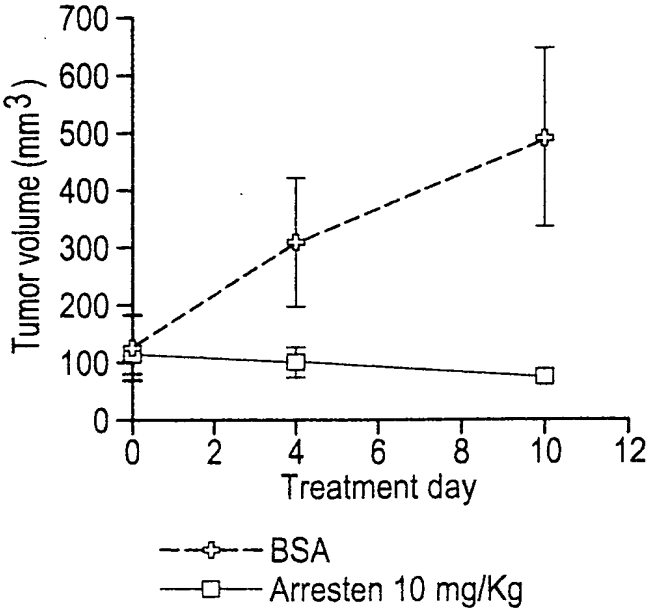


FIG. 9C

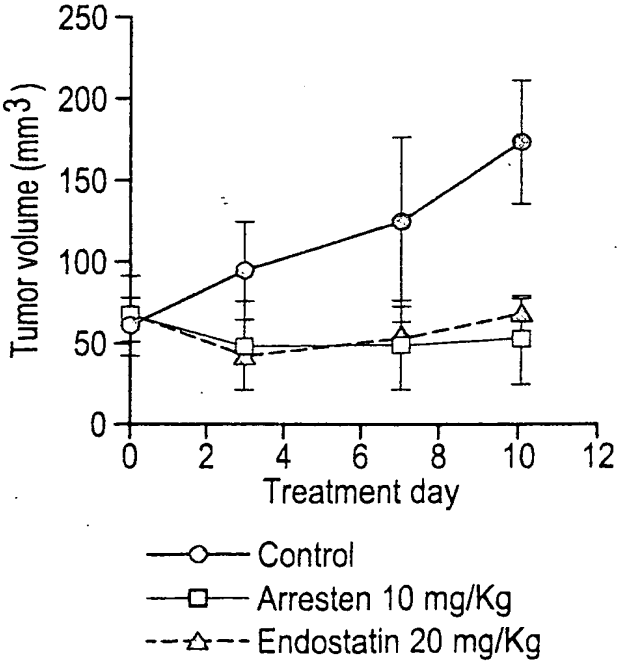
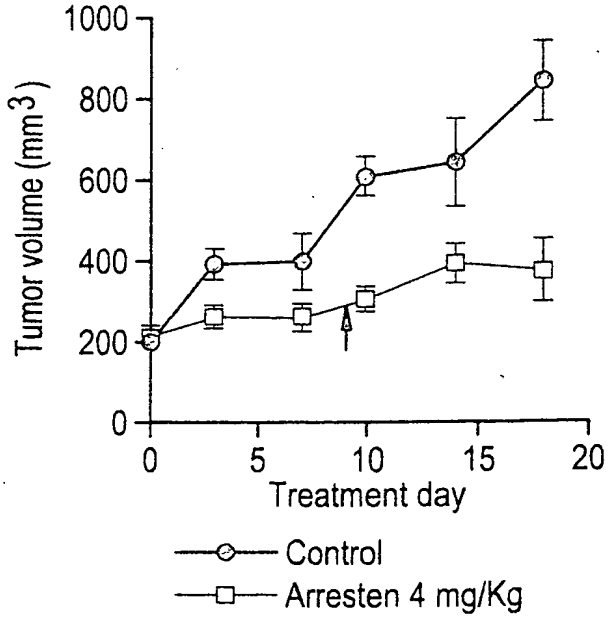


FIG. 9D



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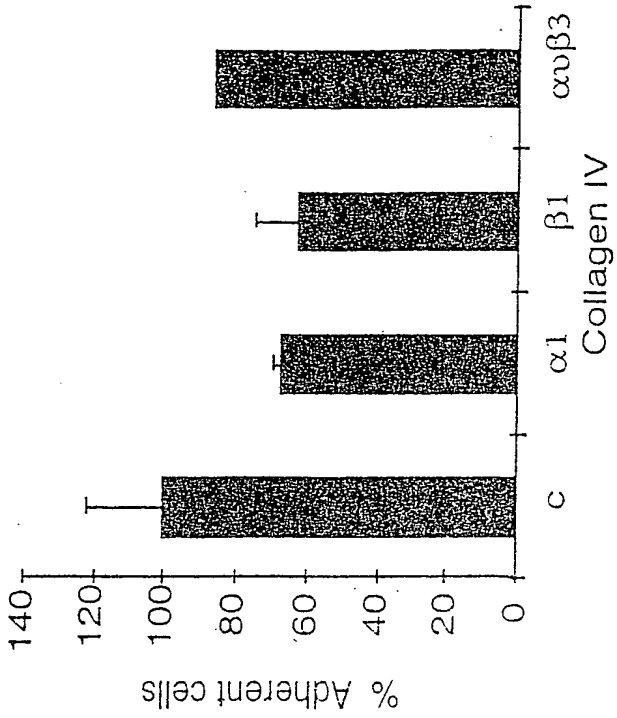

FIG. 10B

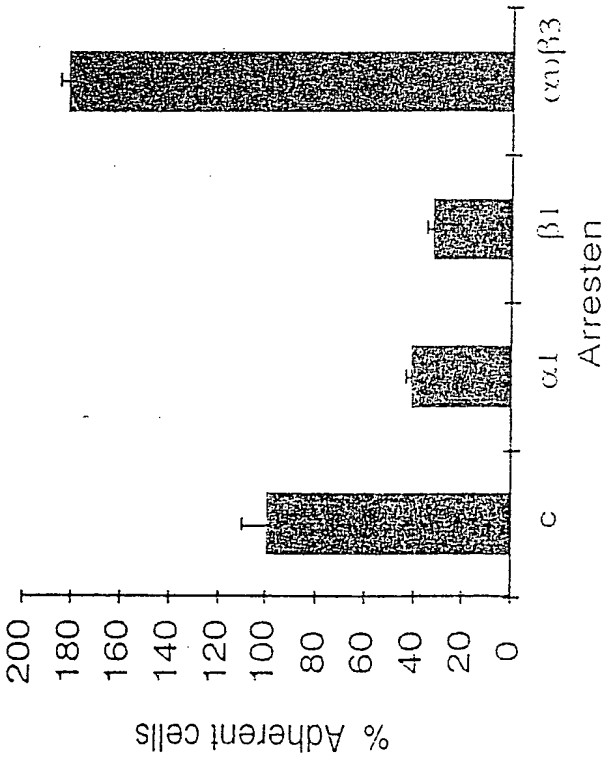

FIG. 10A

FIG. 11A

pET22b(+) forward primer:
5'-CGGGATCCT GTC AGC ATC GGC TAC CTC-3' (SEQ ID NO:7)
pET22b(+) reverse primer:
5'-CCCAAGCTT CAG GTT CTT CAT GCA CAC-3' (SEQ ID NO:8)
pPICZαA forward primer:
5'-TTCGGAATTC GTC AGC ATC GGC TAC CTC CTG-3' (SEQ ID NO:17)
pPICZαA reverse primer:
5'-GGGGTACCCC CAG GTT CTT CAT GCA CAC CTG G-3' (SEQ ID NO:18)

```
      5      10      15      20      25      30      35      40      45
gtc agc atc ggc tac ctc ctg gtg aag cac agc cag acg gac cag

     50      55      60      65      70      75      80      85      90
gag ccc atg tgc cca gtg ggc atg aac aaa ctc tgg agt gga tac

     95     100     105     110     115     120     125     130     135
agc ctg ctg tac ttc gag ggc cag gag aag gcg cac aac cag gac

    140     145     150     155     160     165     170     175     180
ctg ggg ctg gcg ggc tcc tgc ctg gcg cgg ttc agc acc atg ccc

    185     190     195     200     205     210     215     220     225
ttc ctg tac tgc aac cct ggt gat gtc tgc tac tat gcc agc cgg

    230     235     240     245     250     255     260     265     270
aac gac aag tcc tac tgg ctc tct acc act gcg ccg ctg ccc atg

    275     280     285     290     295     300     305     310     315
atg ccc gtg gcc gag gac gag atc aag ccc tac atc agc cgc tgt

    320     325     330     335     340     345     350     355     360
tct gtg tgt gag gcc ccg gcc atc gcc atc gcg gtc cac agt cag

    365     370     375     380     385     390     395     400     405
gat gtc tcc atc cca cac tgc cca gct ggg tgg cgg agt ttg tgg

    410     415     420     425     430     435     440     445     450
atc gga tat tcc ttc ctc atg cac acg gcg gcg gga gac gaa ggc

    455     460     465     470     475     480     485     490     495
gtg ggc caa tca ctg gtg tca ccg ggc agc tgt cta gag gac ttc

    500     505     510     515     520     525     530     535     540
cgc gcc aca cca ttc atc gaa tgc aat gga ggc cgc ggc acc tgc

    545     550     555     560     565     570     575     580     585
cac tac tac gcc aac aag tac agc ttc tgg ctg acc acc att ccc

    590     595     600     605     610     615     620     625     630
gag cag agc ttc cag ggc tcg ccc tcc gcc gac acg ctc aag gcc

    635     640     645     650     655     660     665     670     675
ggc ctc atc cgc aca cac atc agc cgc tgc cag gtg tgc atg aag

    680
aac ctg tga      (SEQ ID NO:5)
```

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FIG. 11B

5 10 15 20 25 30 35 40 45
VSI GYL LVK HSQ TDQ VSI GYL LVK HSQ TDQ EPM CPV GMN KLW SGY

50 55 60 65 70 75 80 85 90
SLL YFE GQE KAH NQD LGL AGS CLA RFS TMP FLY CNP GDV CYY ASR

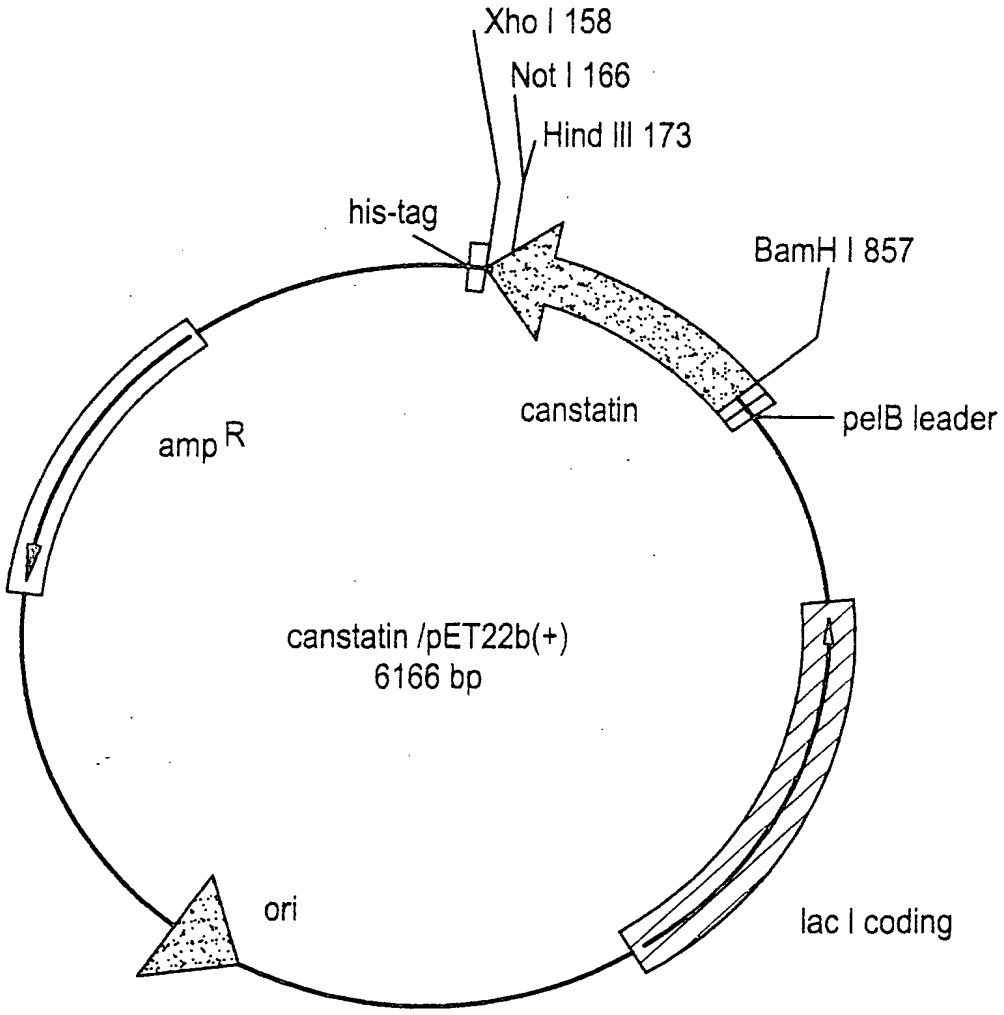
95 100 105 110 115 120 125 130 135
NDK SYW LST TAP LPM MPV AED EIK PYI SRC SVC EAP AIA IAV HSQ

140 145 150 155 160 165 170 175 180
DVS IPH CPA GWR SLW IGY SFL MHT AAG DEG GGQ SLV SPG SCL EDF

185 190 195 200 205 210 215 220 225
RAT PFI ECN GGR GTC HYY ANK YSF WLT TIP EQS FQG SPS ADT LKA

230 235 240
GLI RTH ISR CQV CMK NL (SEQ ID NO:6)

FIG. 12



Forward primer: 5'-cgggatcctgtcagcatcggctacctc-3'

Reverse primer: 5'-cccaagcttcaggttcttcatgcacac-3'

FIG. 13A

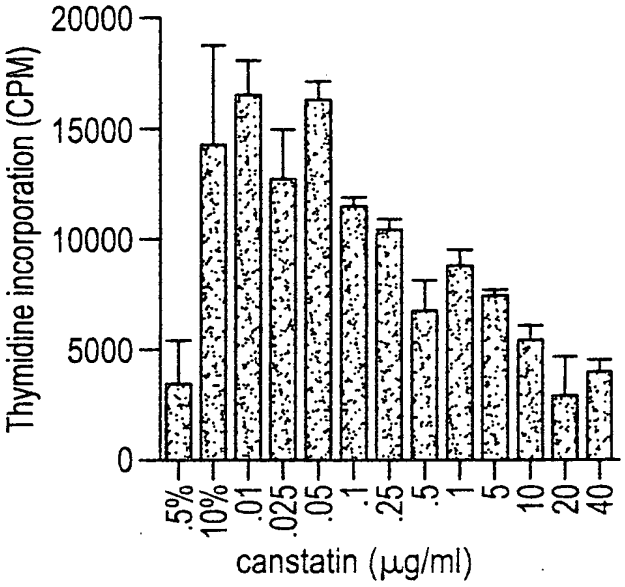


FIG. 13B

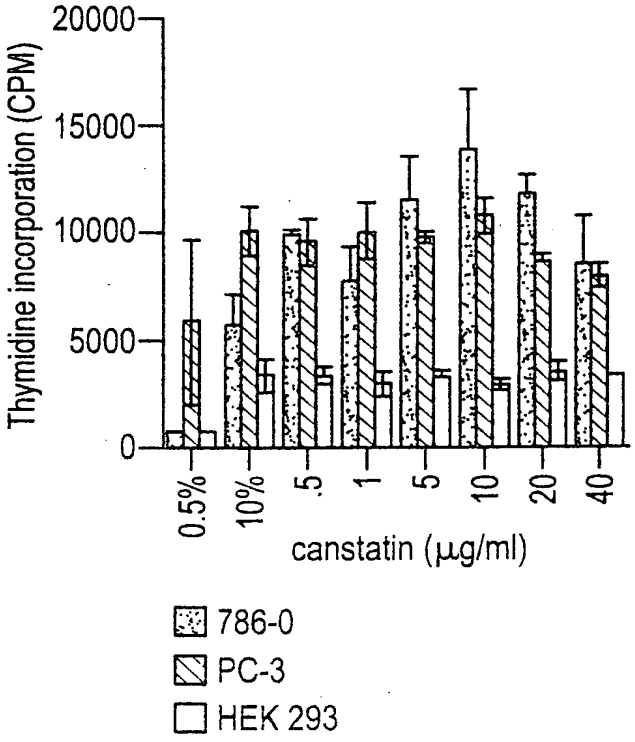


FIG. 13C

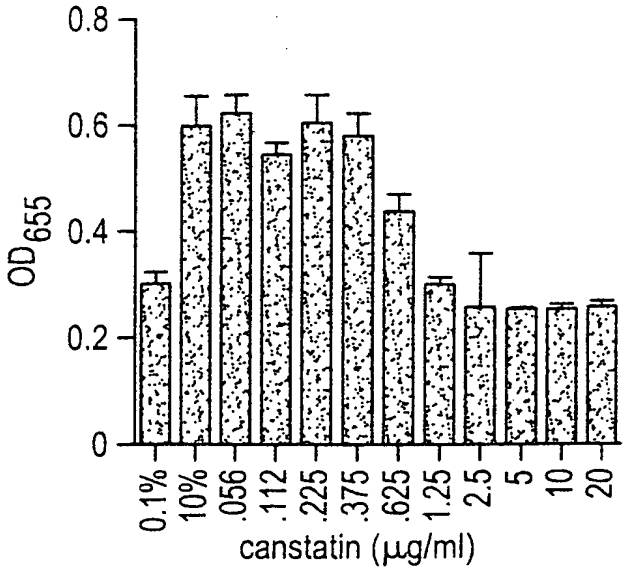


FIG. 13D

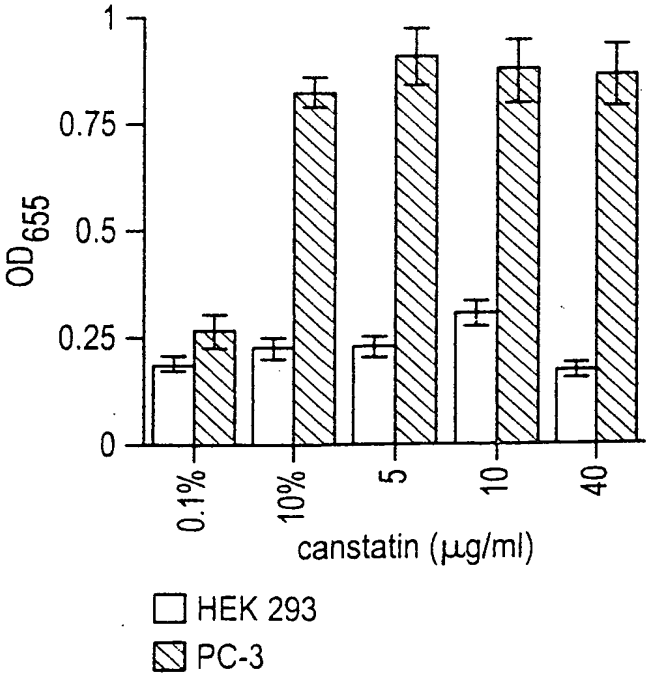


FIG. 14

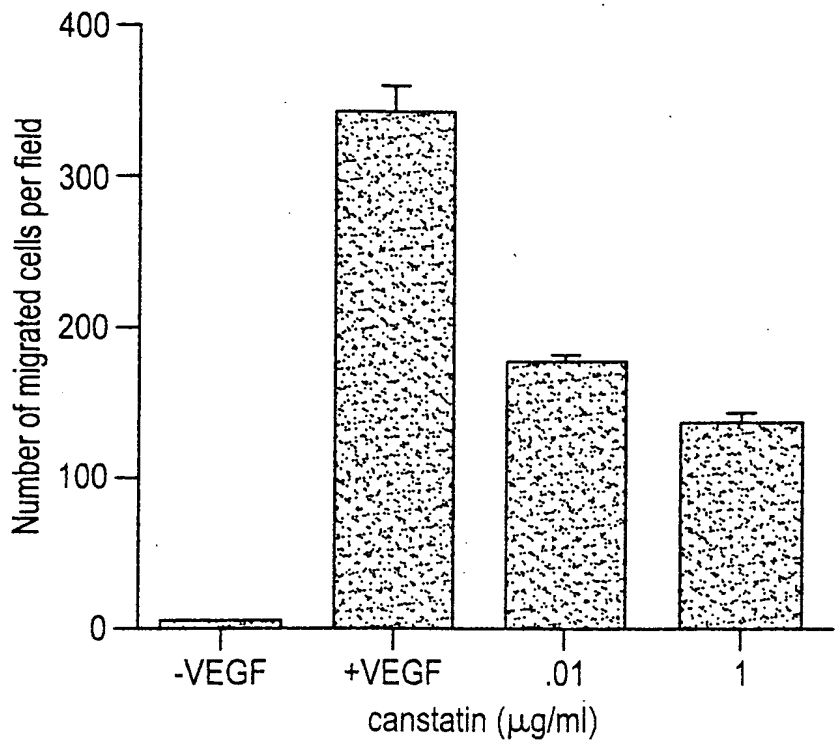

400
300
200
100
0
Number of migrated cells per field
-VEGF
+VEGF
.01
1
canstatin (μg/ml)

FIG. 15

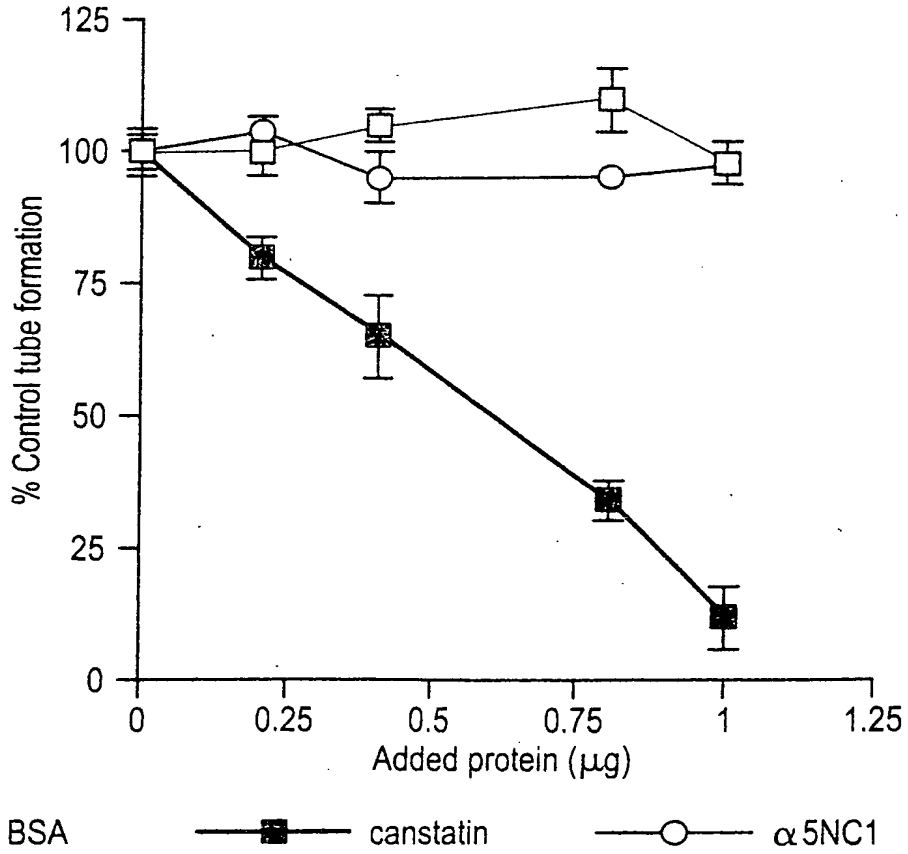

125
100
75
50
25
0
% Control tube formation
0
0.25
0.5
0.75
1
1.25
Added protein (μg)
BSA
canstatin
α5NC1

FIG. 16

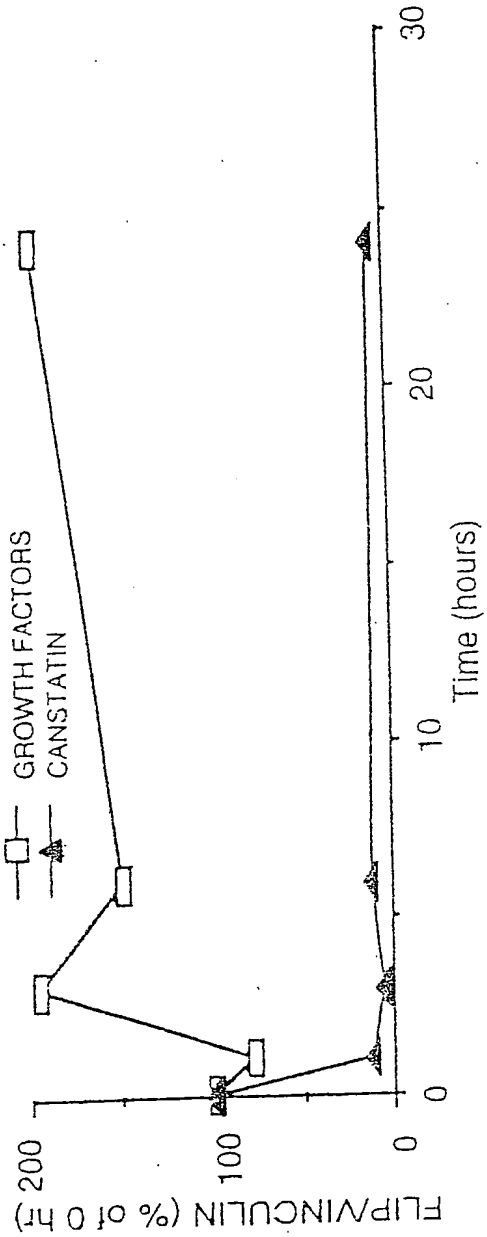


FIG. 17A

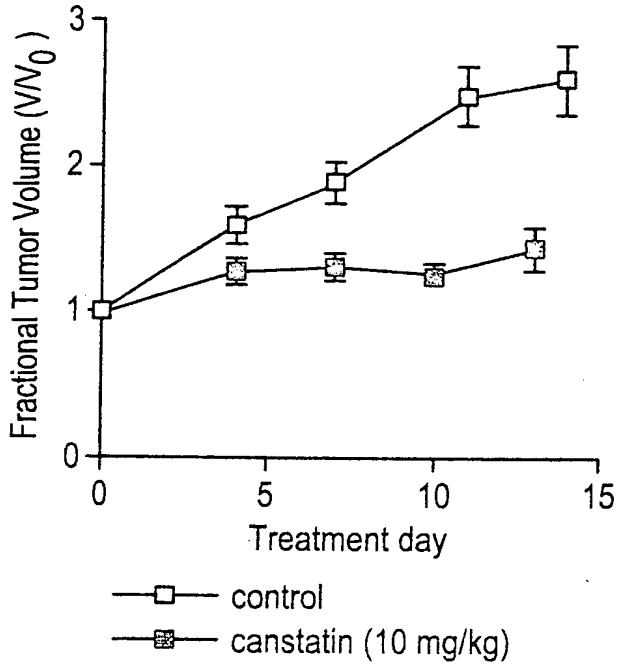


FIG. 17B

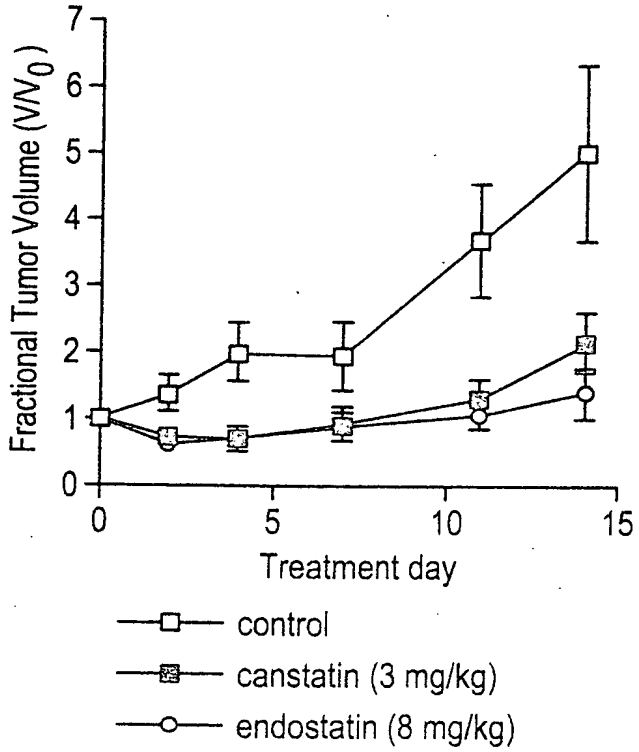


FIG. 17C

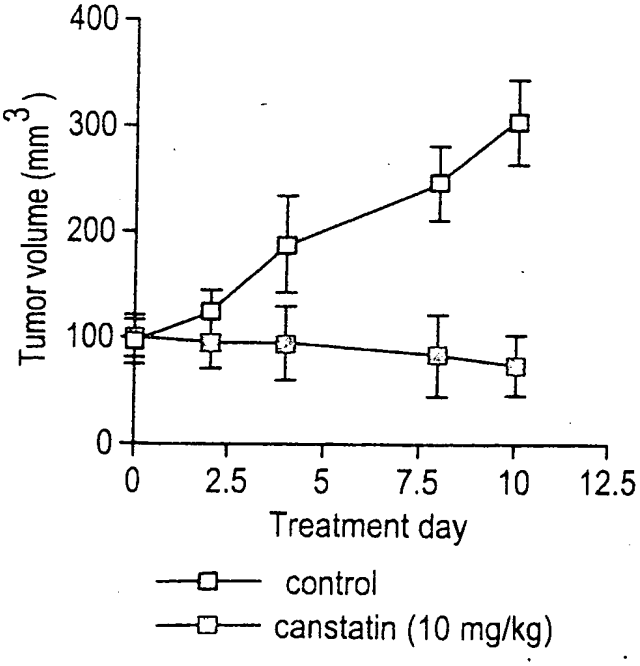


FIG. 17D

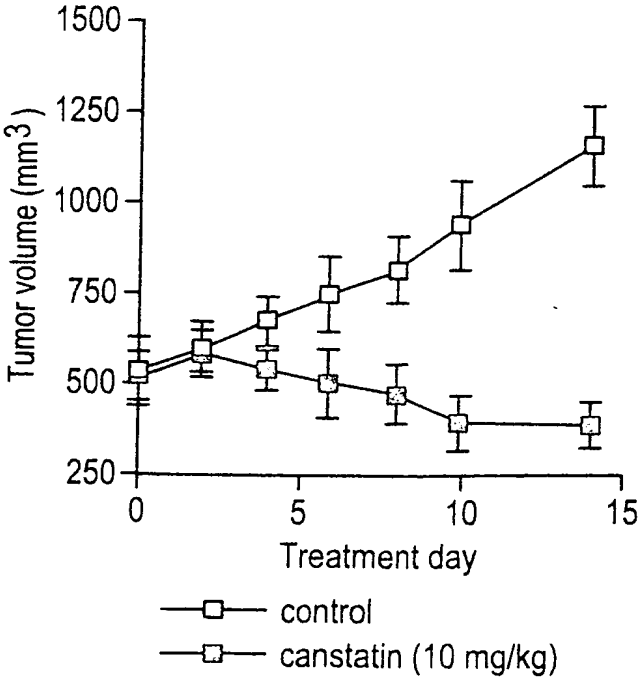


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FIG. 18A

pET22b(+) forward primer:

5'-CGGGAT <u>CCA GGT TTG AAA GGA AAA CGT</u>-3' (SEQ ID NO:11)

pET22b(+) reverse primer:

5'-CCCAAGCTT <u>TCA GTG TCT TTT CTT CAT</u>-3' (SEQ ID NO:12)

```
  5   10  15   20   25  30   35   40  45
cca ggt ttg aaa gga aaa cgt gga gac agt gga tca cct gca acc

 50   55   60   65   70   75   80   85   90
tgg aca acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca

 95  100  105  110  115  120  125  130  135
gca att cct tca tgt cca gag ggg aca gtg cca ctc tac agt ggg

140  145  150  155  160  165  170  175  180
ttt tct ttt ctt ttt gta caa gga aat caa cga gcc cac gga caa

185  190  195  200  205  210  215  220  225
gac ctt gga act ctt ggc agc tgc ctg cag cga ttt acc aca atg

230  235  240  245  250  255  260  265  270
cca ttc tta ttc tgc aat gtc aat gat gta tgt aat ttt gca tct

275  280  285  290  295  300  305  310  315
cga aat gat tat tca tac tgg ctg tca aca cca gct ctg atg cca

320  325  330  335  340  345  350  355  360
atg aac atg gct ccc att act ggc aga gcc ctt gag cct tat ata

365  370  375  380  385  390  395  400  405
agc aga tgc act gtt tgt gaa ggt cct gcg atc gcc ata gcc gtt

410  415  420  425  430  435  440  445  450
cac agc caa acc act gac att cct cca tgt cct cac ggc tgg att

455  460  465  470  475  480  485  490  495
tct ctc tgg aaa gga ttt tca ttc atc atg ttc aca agt gca ggt

500  505  510  515  520  525  530  535  540
tct gag ggc acc ggg caa gca ctg gcc tcc cct ggc tcc tgc ctg

545  550  555  560  565  570  575  580  585
gaa gaa ttc cga gcc agc cca ttt cta gaa tgt cat gga aga gga

590  595  600  605  610  615  620  625  630
acg tgc aac tac tat tca aat tcc tac agt ttc tgg ctg gct tca

635  640  645  650  655  660  665  670  675
tta aac cca gaa aga atg ttc aga aag cct att cca tca act gtg

680  685  690  695  700  705  710  715  720
aaa gct ggg gaa tta gaa aaa ata ata agt cgc tgt cag gtg tgc

725  730  735
atg aag aaa aga cac tga        (SEQ ID NO:9)
```

pET22b-α3(IV) NC1 = nucleotides 4 through 735

Tumstatin 333 = nucleotides 4 through 375

Tumstatin 334 - nucleotide 376 through 735

FIG. 18B

```
 *
     5      10     15      20      25     30      35      40     45
PGL KGK RGD SGS PAT WTT RGF VFT RHS QTT AIP SCP EGT VPL YSG

    50      55     60      65      70     75      80      85     90
FSF LFV QGN QRA HGQ DLG TLG SCL QRF TTM PFL FCN VND VCN FAS

                                              *+
    95     100    105     110     115    120     125     130    135
RND YSY WLS TPA LMP MNM API TGR ALE PYI SRC TVC EGP AIA IAV

   140     145    150     155     160    165     170     175    180
HSQ TTD IPP CPH GWI SLW KGF SFI MFT SAG SEG TGQ ALA SPG SCL

   185     190    195     200     205    210     215     220    225
EEF RAS PFL ECH GRG TCN YYS NSY SFW LAS LNP ERM FRK PIP STV

                           +
   230     235    240     245
KAG ELE KII SRC QVC MKK RH      (SEQ ID NO:10)
```

pET22b α3(IV) NC1 = residues 2 through 245
Tumstatin 333 = residues 2 through 125
Tumstatin 334 = residues 126 through 245

FIG. 19

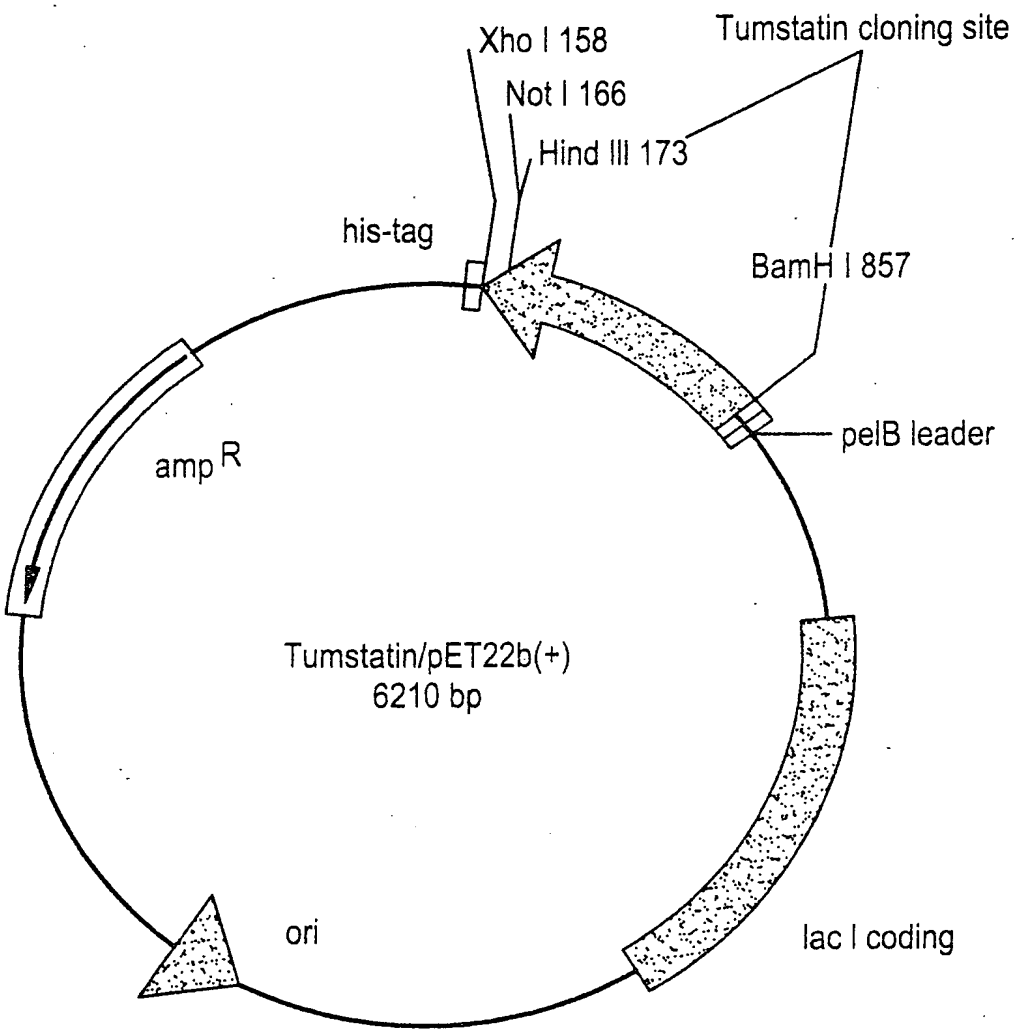

Xho I 158
Not I 166
Hind III 173
Tumstatin cloning site
his-tag
BamH I 857
pelB leader
amp R
Tumstatin/pET22b(+)
6210 bp
ori
lac I coding

FIG. 20

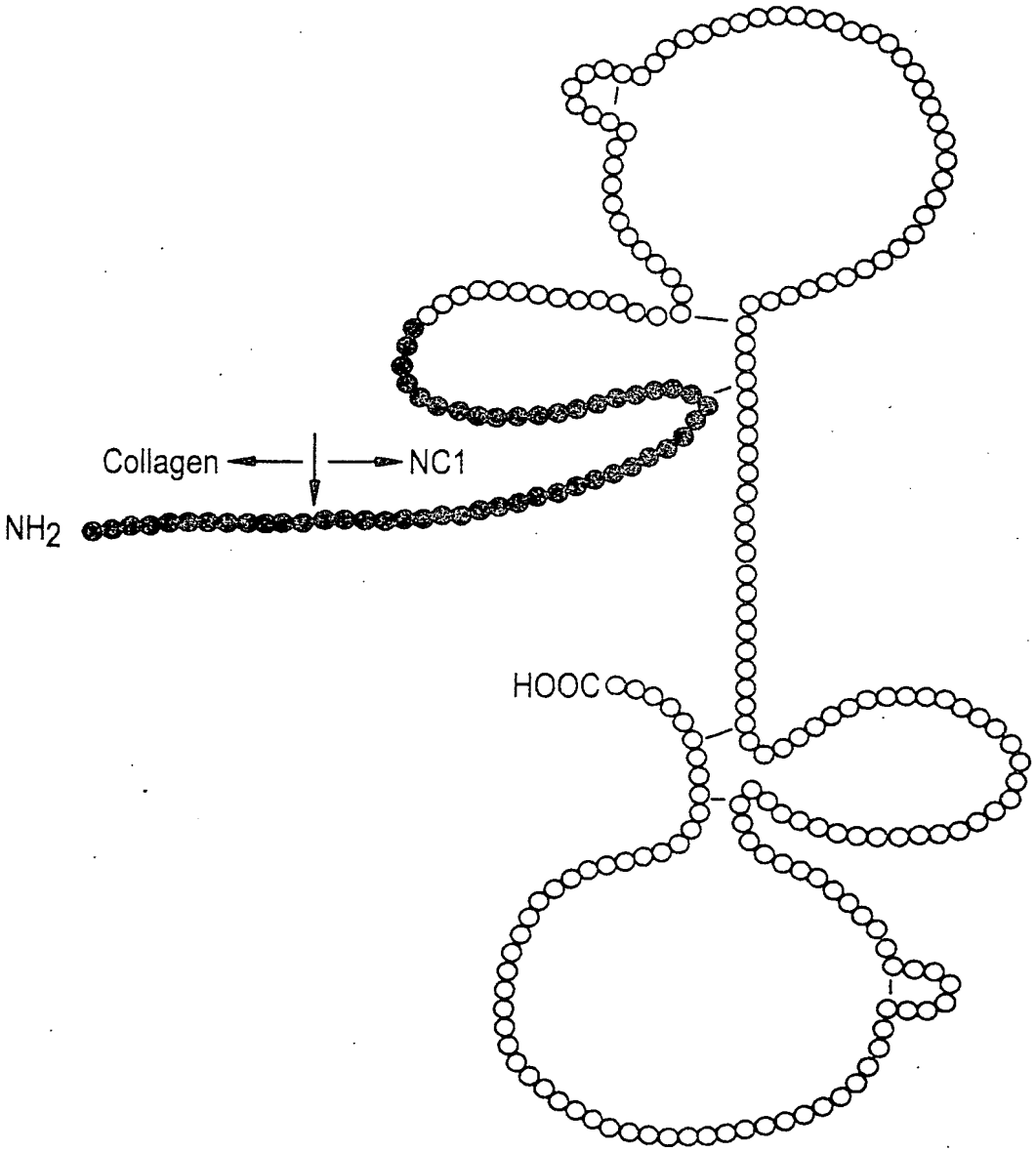
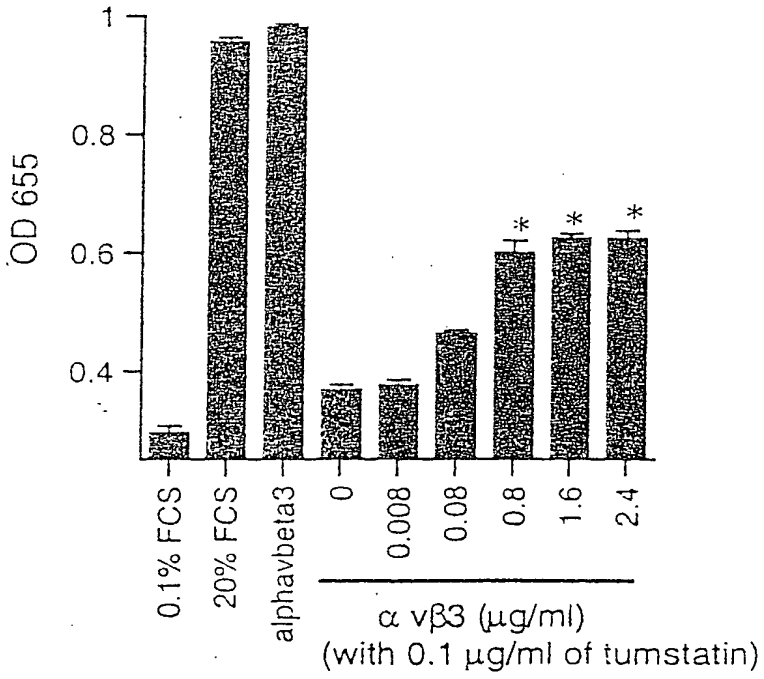


FIG. 21C

FIG. 21B

FIG. 21A

FIG. 22


OD 655
1
0.8
0.6
0.4
*
*
*
0.1% FCS
20% FCS
alphavbeta3
0
0.008
0.08
0.8
1.6
2.4
α vβ3 (μg/ml)
(with 0.1 μg/ml of tumstatin)

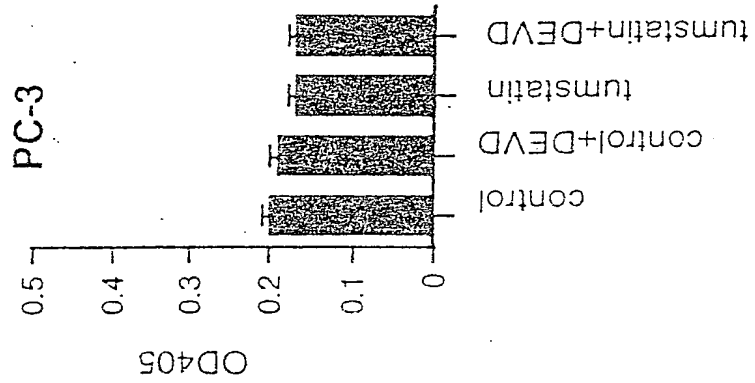


FIG. 23B

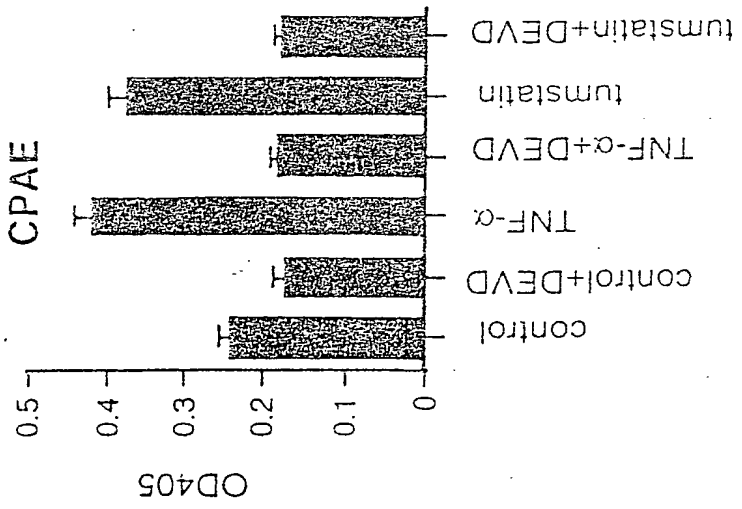


FIG. 23A

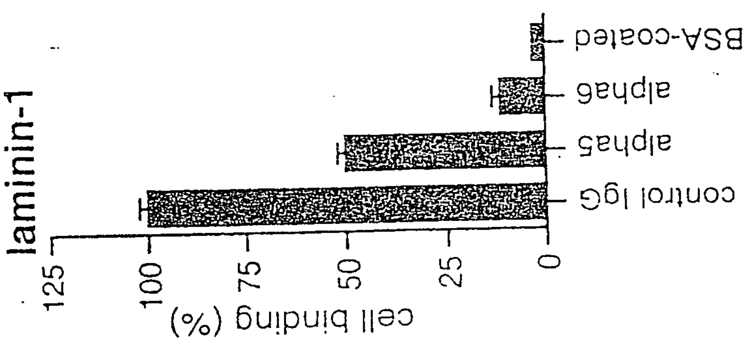


FIG. 24D

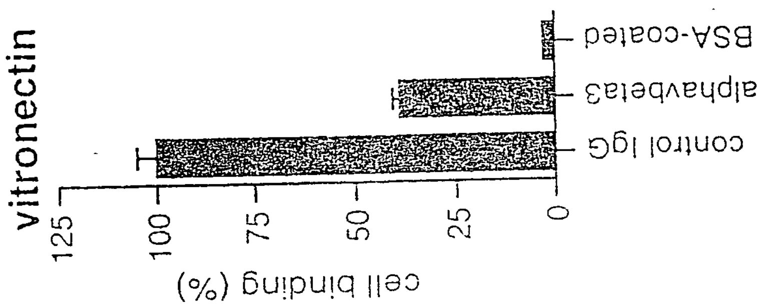


FIG. 24C

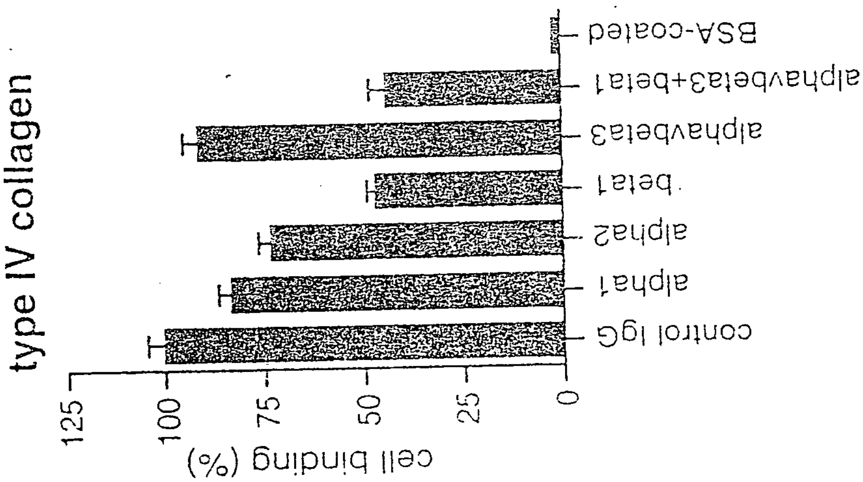


FIG. 24B

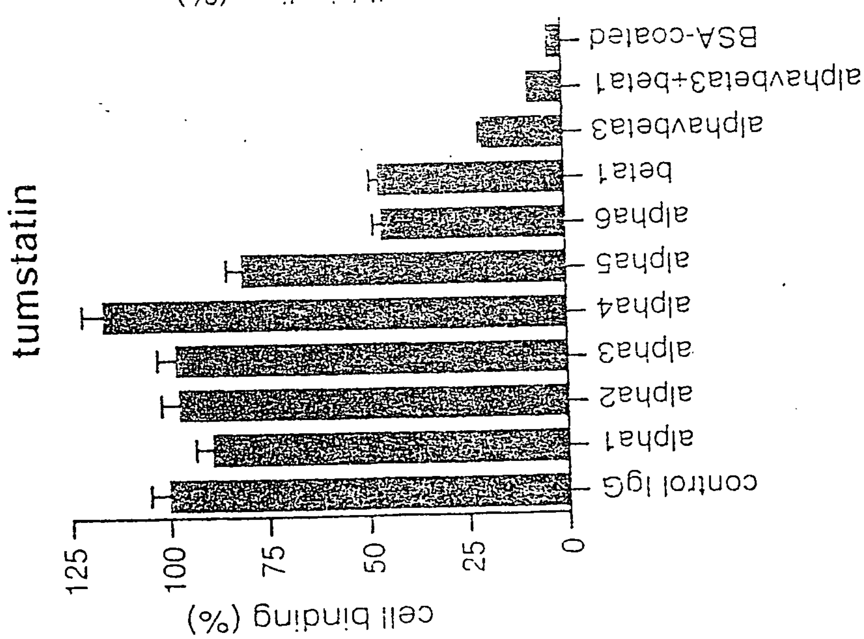
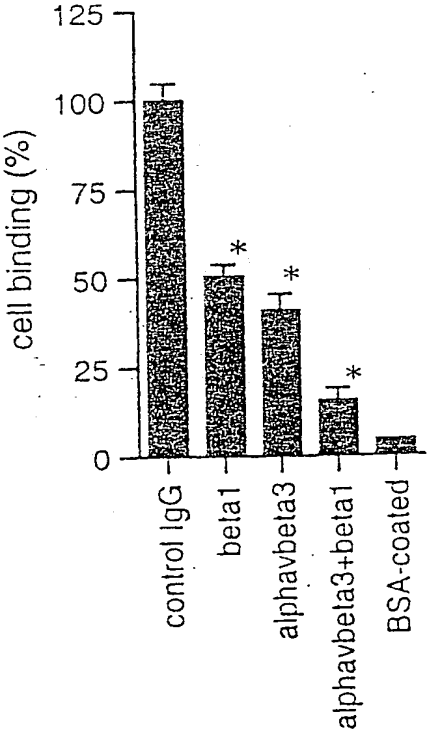


FIG. 24A

FIG. 25


125
100
75
50
25
0
cell binding (%)
control IgG
beta1
alphavbeta3
alphavbeta3+beta1
BSA-coated
*
*
*

1440,1027-005

FIG. 27B

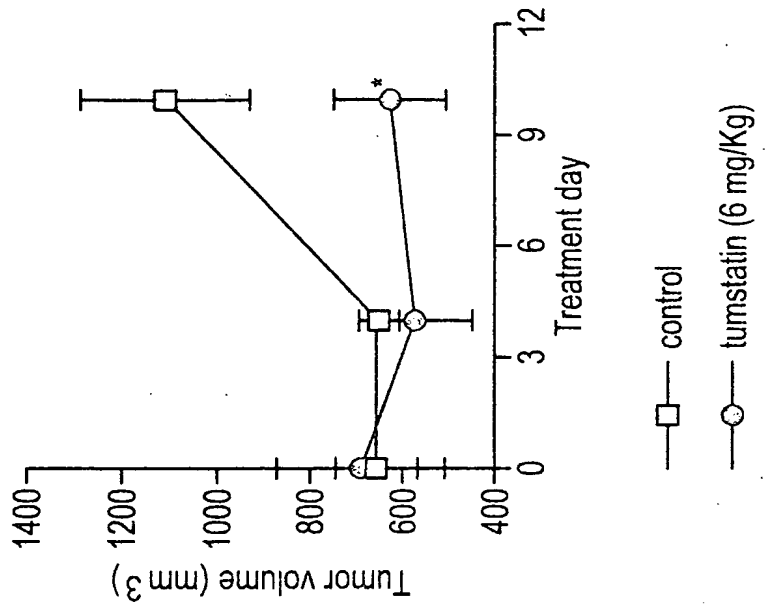


FIG. 27A

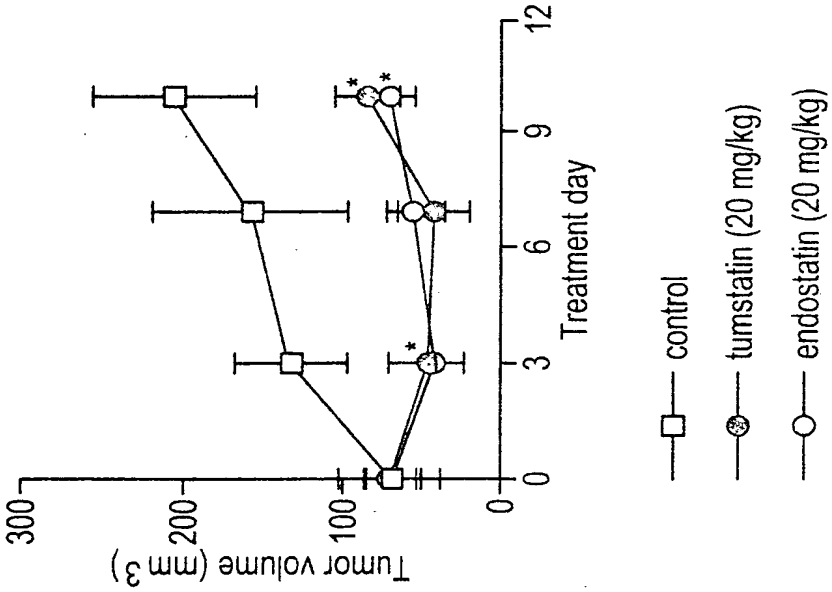


FIG. 26

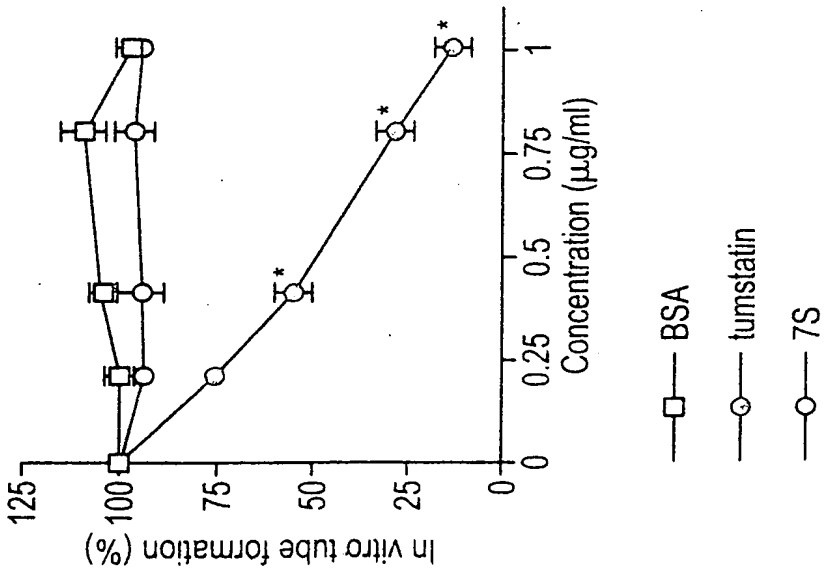
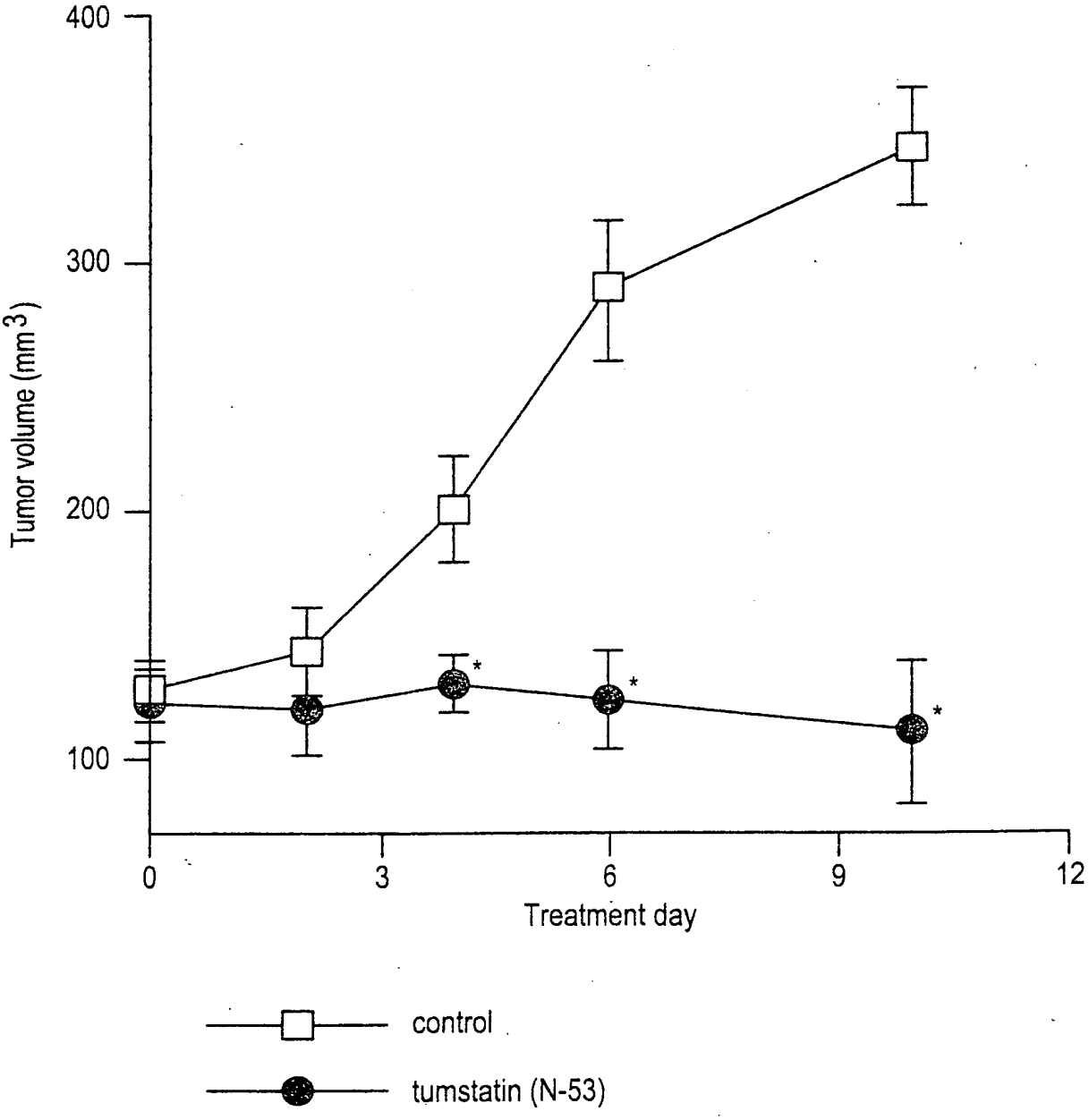


FIG. 28


400
300
200
100
Tumor volume (mm3)
0
3
6
9
12
Treatment day
*
*
*
control
tumstatin (N-53)

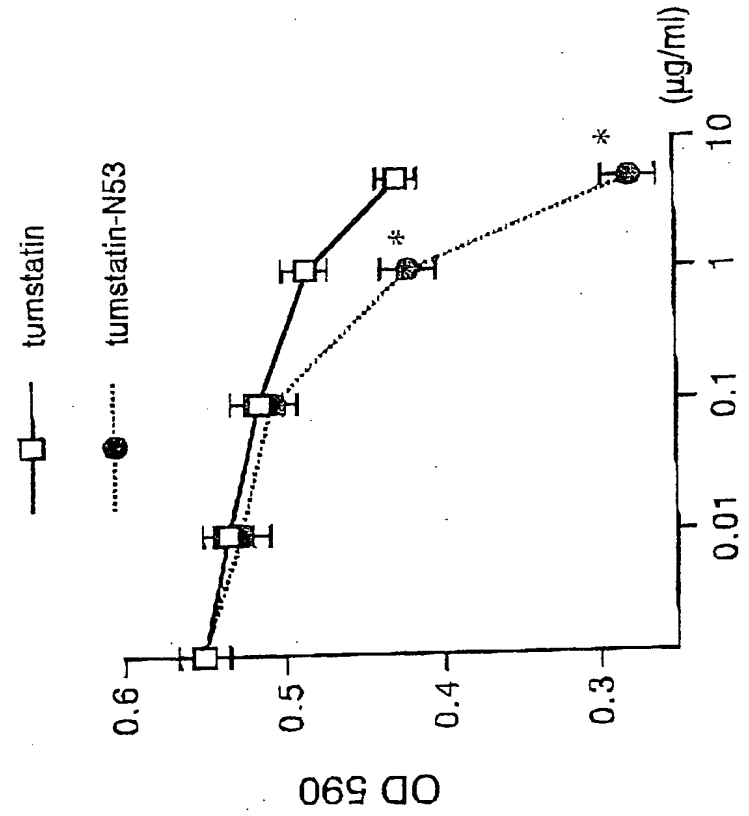


FIG. 29

FIG. 30

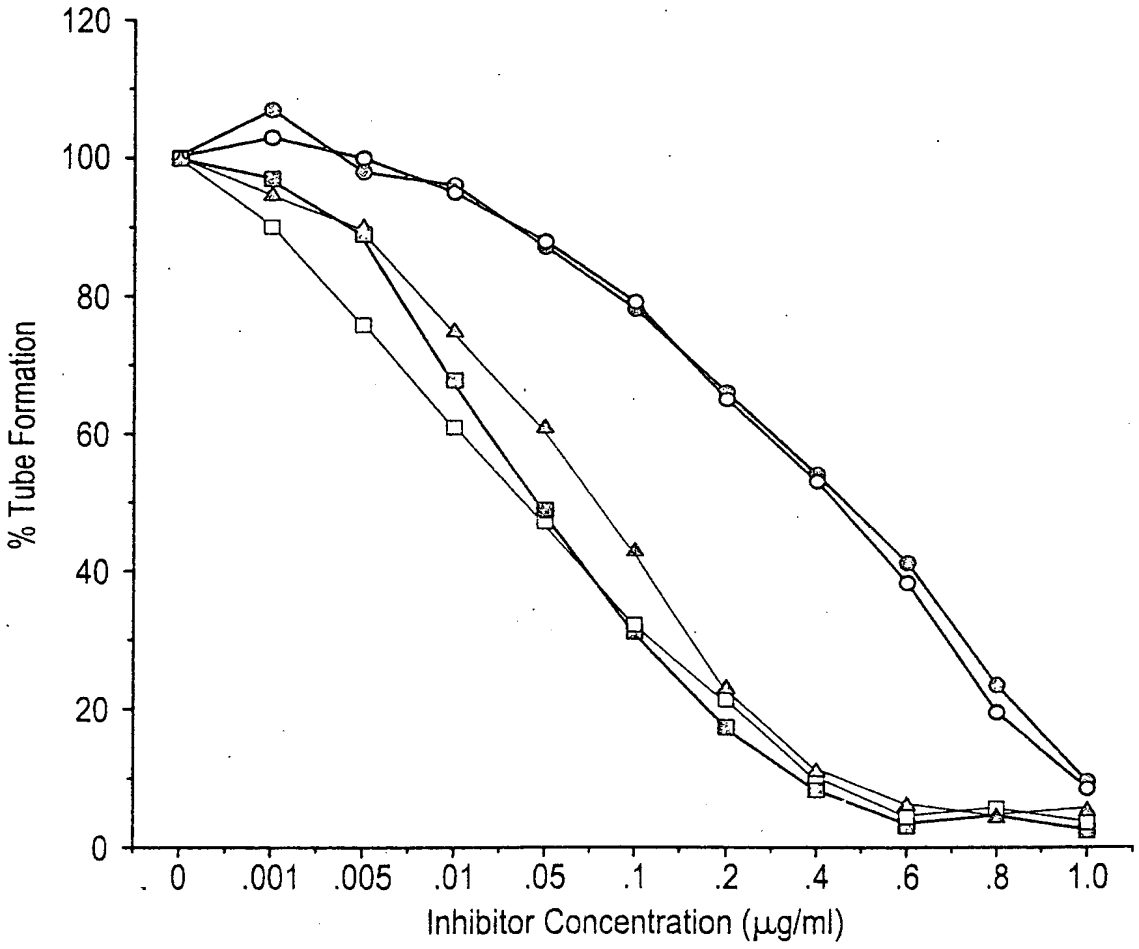
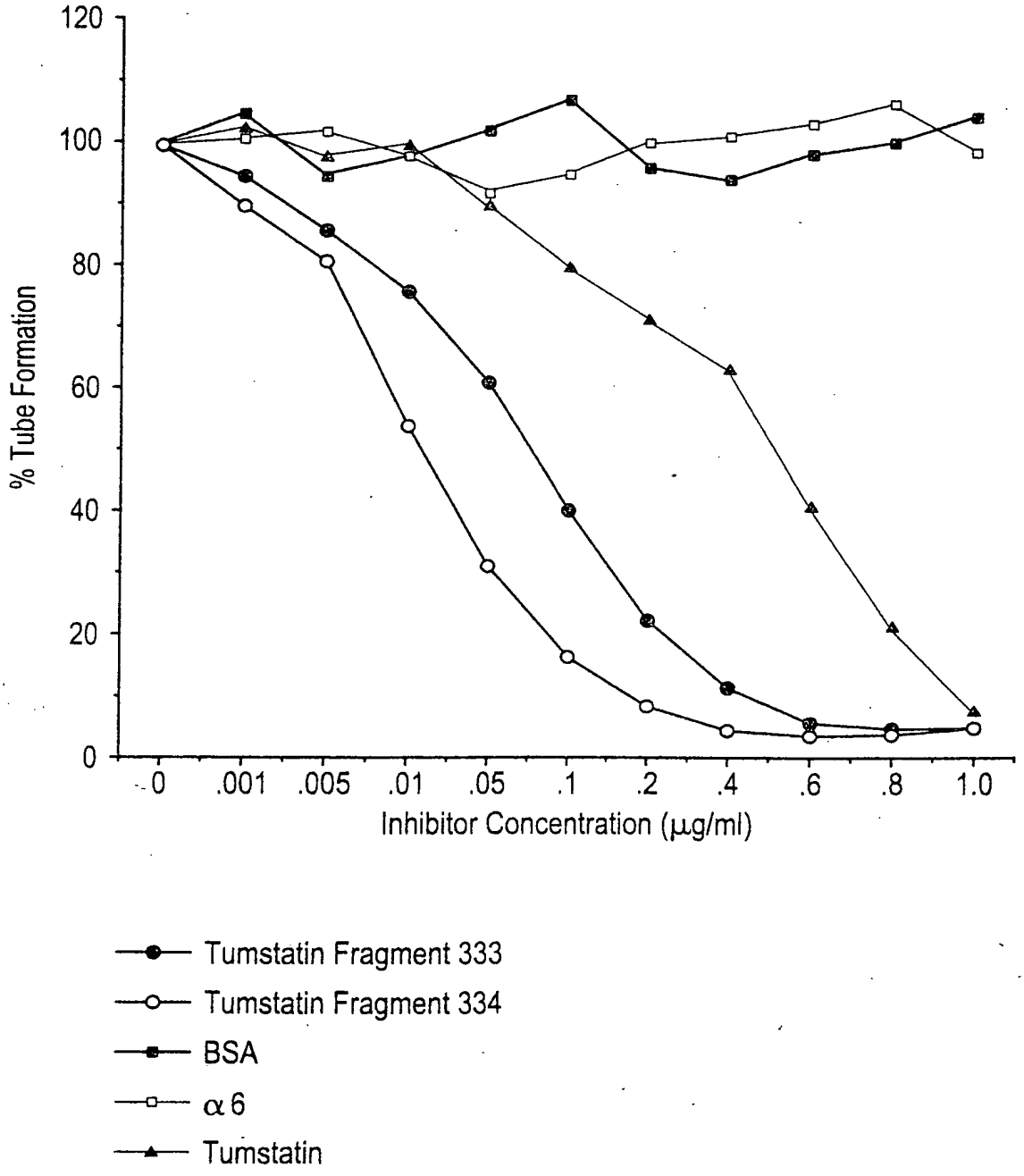
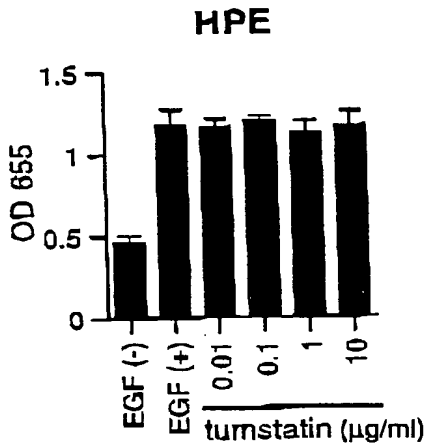

120
100
80
60
40
20
0
% Tube Formation
0
.001
.005
.01
.05
.1
.2
.4
.6
.8
1.0
Inhibitor Concentration (μg/ml)
Arresten
Canstatin
12 kDa fragment of Arresten
8 kDa fragment of Arresten
10 kDa fragment of Canstatin

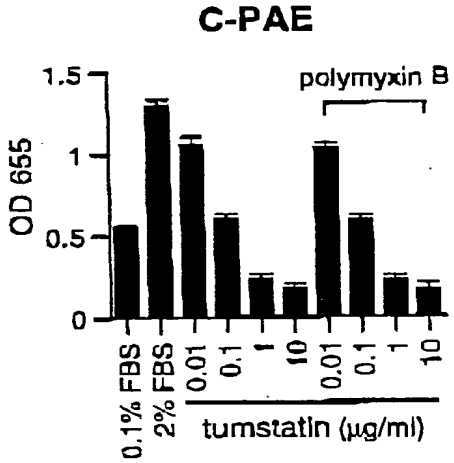
FIG. 31


120
100
80
60
40
20
0
% Tube Formation
0
.001
.005
.01
.05
.1
.2
.4
.6
.8
1.0
Inhibitor Concentration (μg/ml)
Tumstatin Fragment 333
Tumstatin Fragment 334
BSA
α 6
Tumstatin

A


HPE
OD 655
1.5
1
0.5
0
EGF (-)
EGF (+)
0.01
0.1
1
10
tumstatin (μg/ml)

B


C-PAE
polymyxin B
OD 655
1.5
1
0.5
0
0.1% FBS
2% FBS
0.01
0.1
1
10
0.01
0.1
1
10
tumstatin (μg/ml)

C

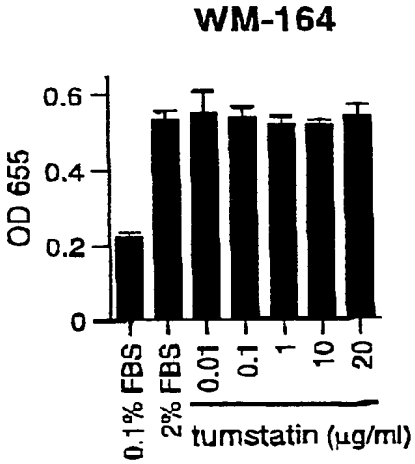

WM-164
OD 655
0.6
0.4
0.2
0
0.1% FBS
2% FBS
0.01
0.1
1
10
20
tumstatin (μg/ml)

Fig. 32

FIG 33((A)

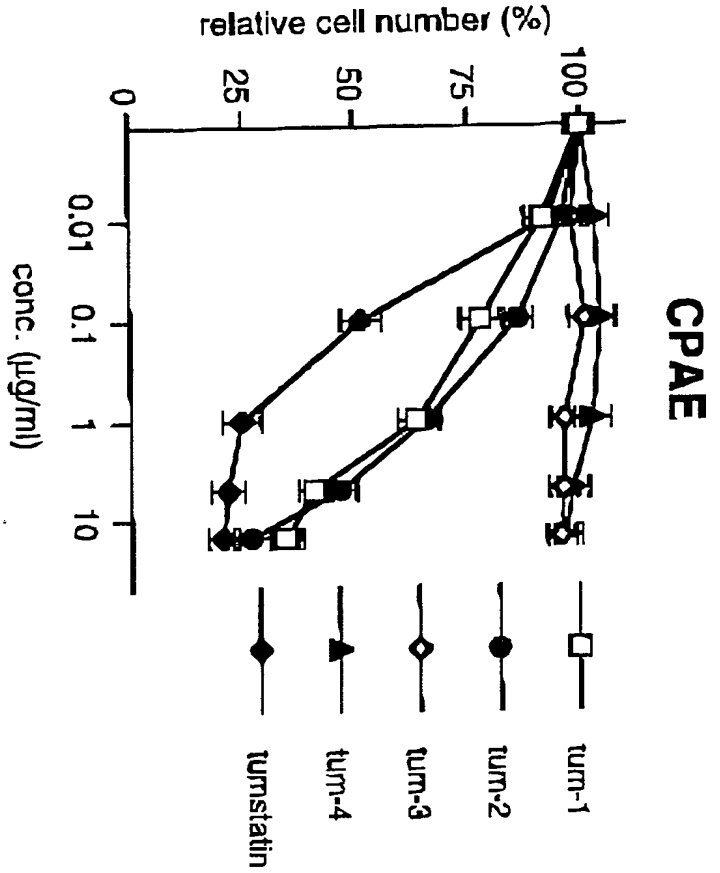


FIG 33 (B)

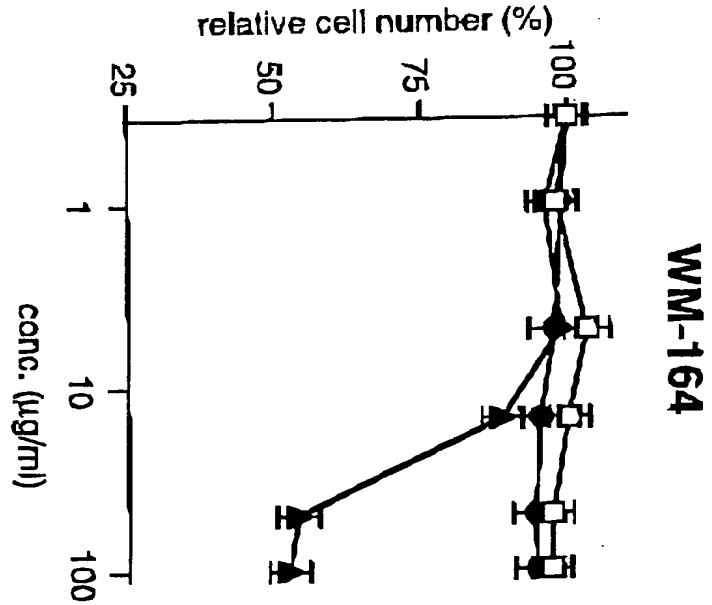
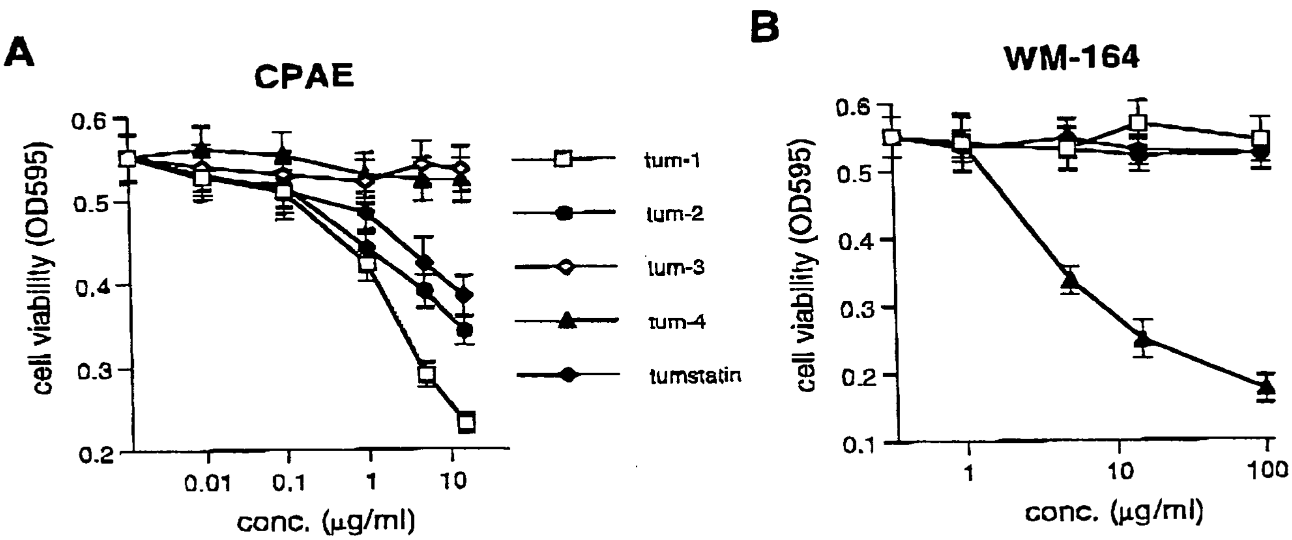


Fig. 34


A
CPAE
cell viability (OD595)
0.6
0.5
0.4
0.3
0.2
0.01
0.1
1
10
conc. (µg/ml)
tum-1
tum-2
tum-3
tum-4
tumstatin
B
WM-164
cell viability (OD595)
0.6
0.5
0.4
0.3
0.2
0.1
1
10
100
conc. (µg/ml)

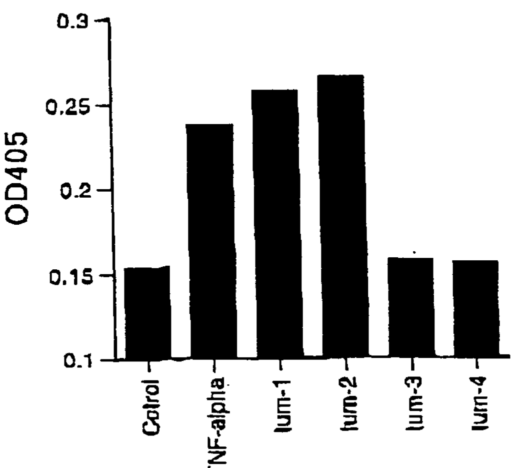

OD405
0.3
0.25
0.2
0.15
0.1
Cotrol
TNF-alpha
tum-1
tum-2
tum-3
tum-4

Fig. 35

1440.1027-005

FIG 36 (A)

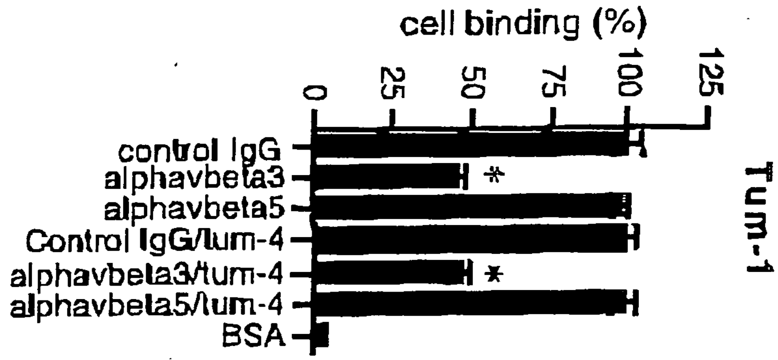


FIG 36 (B)

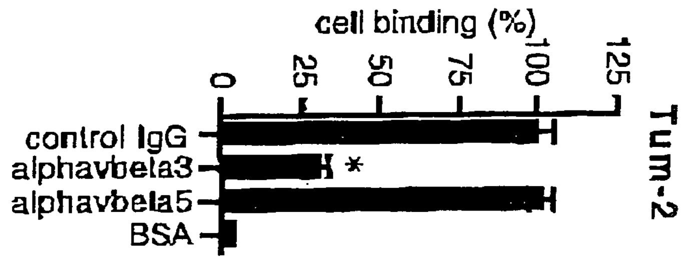


FIG 36 (C)

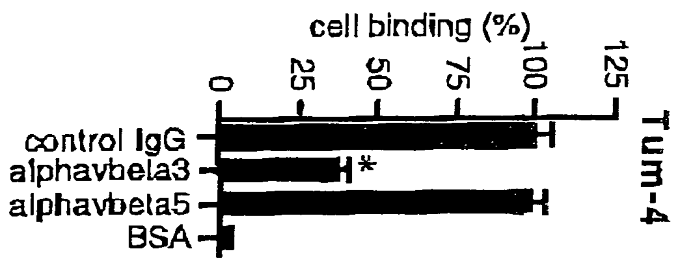
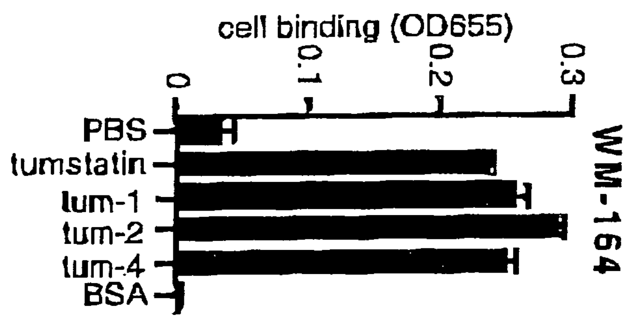


FIG 37



1440.1027-005

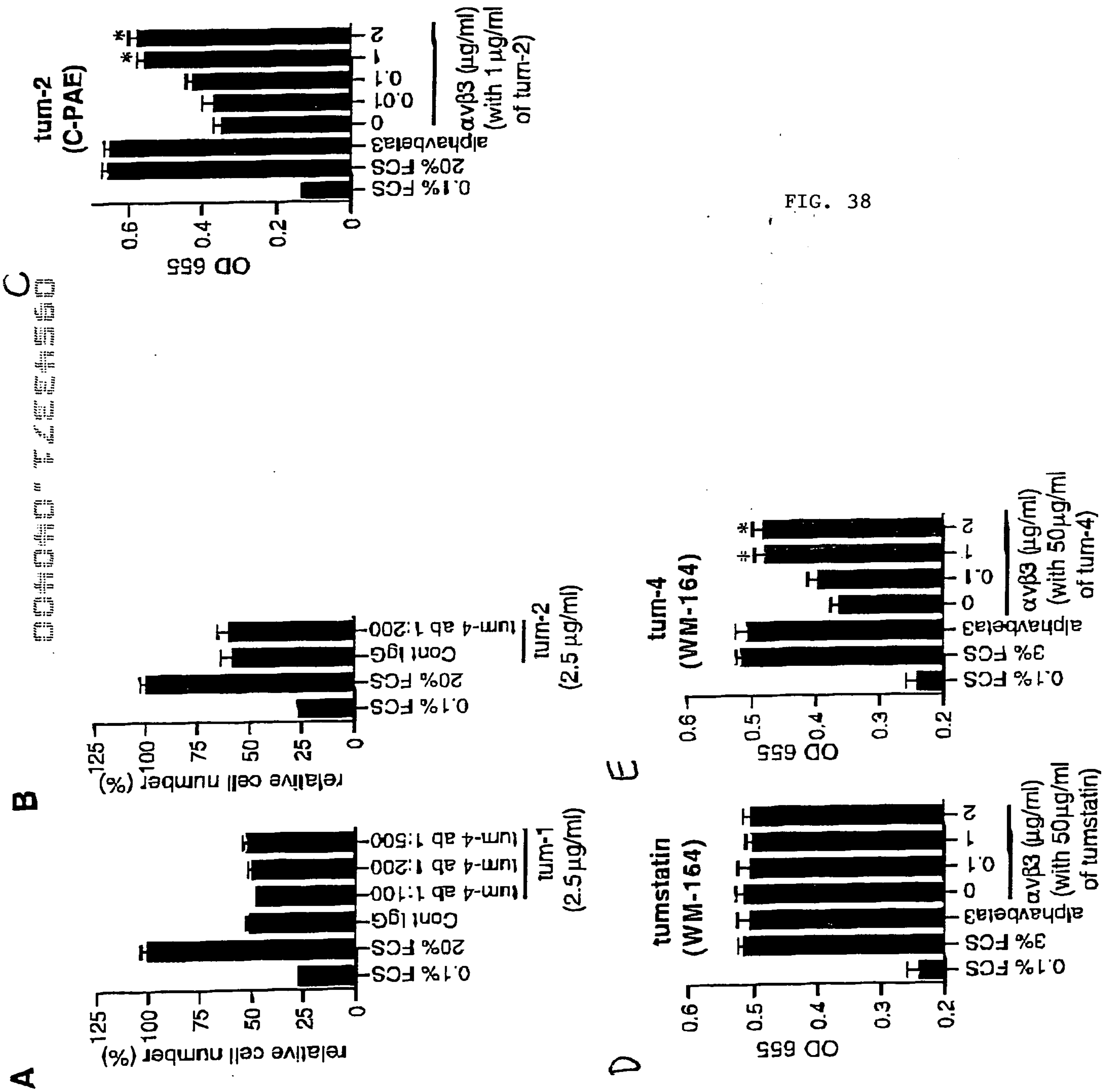
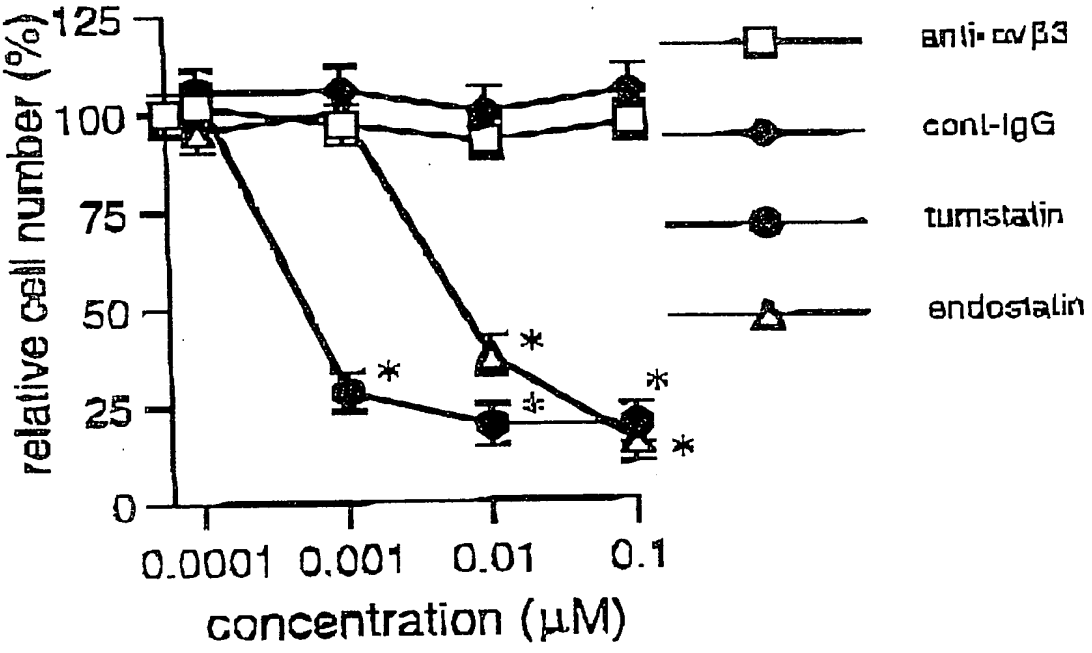


FIG. 38

Fig. 39


relative cell number (%)
125
100
75
50
25
0
0.0001
0.001
0.01
0.1
concentration (μM)
anti-αvβ3
cont-IgG
tumstatin
endostatin
*

1440.1027-005

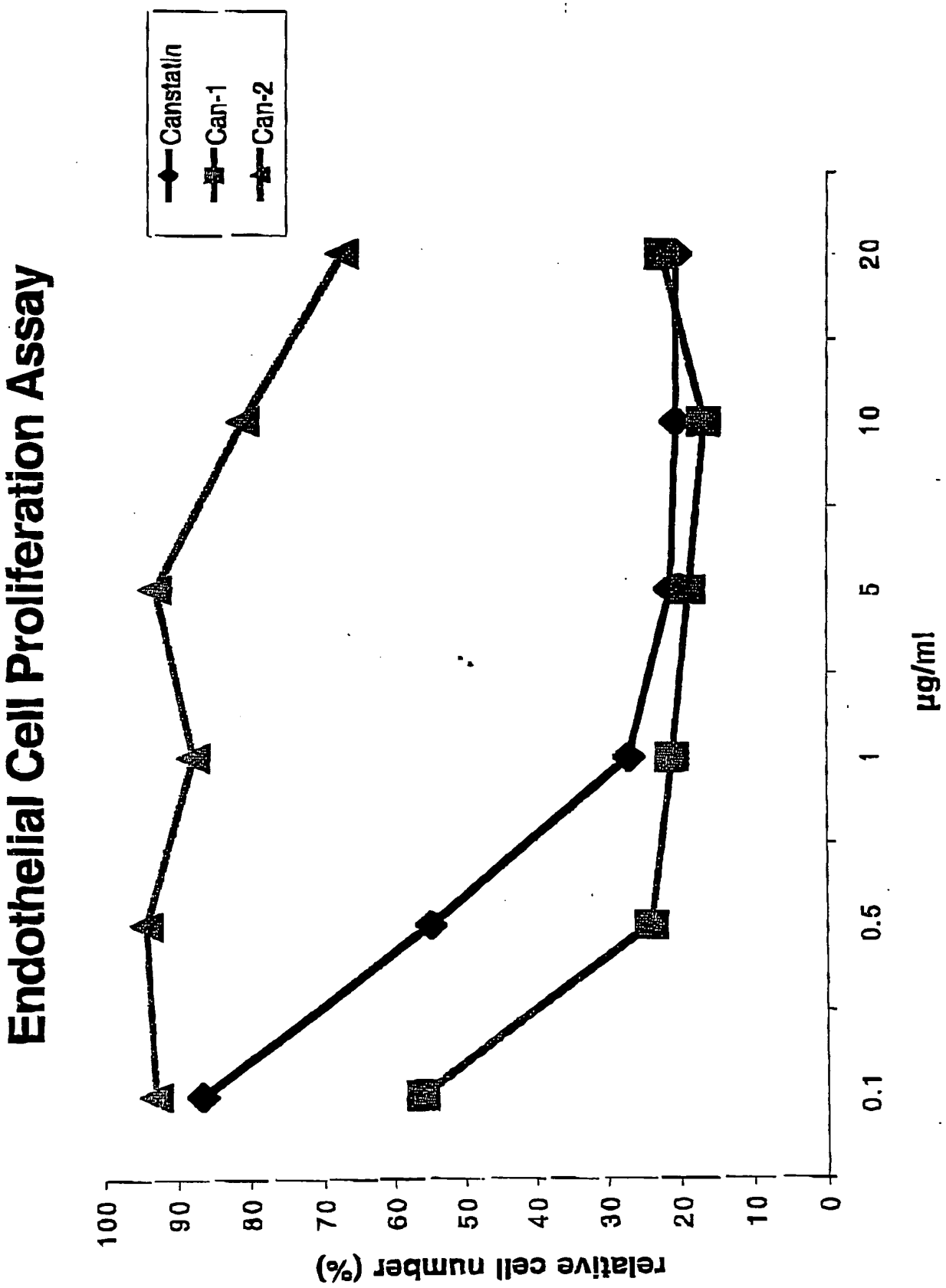


Fig. 40

1440.1027-005

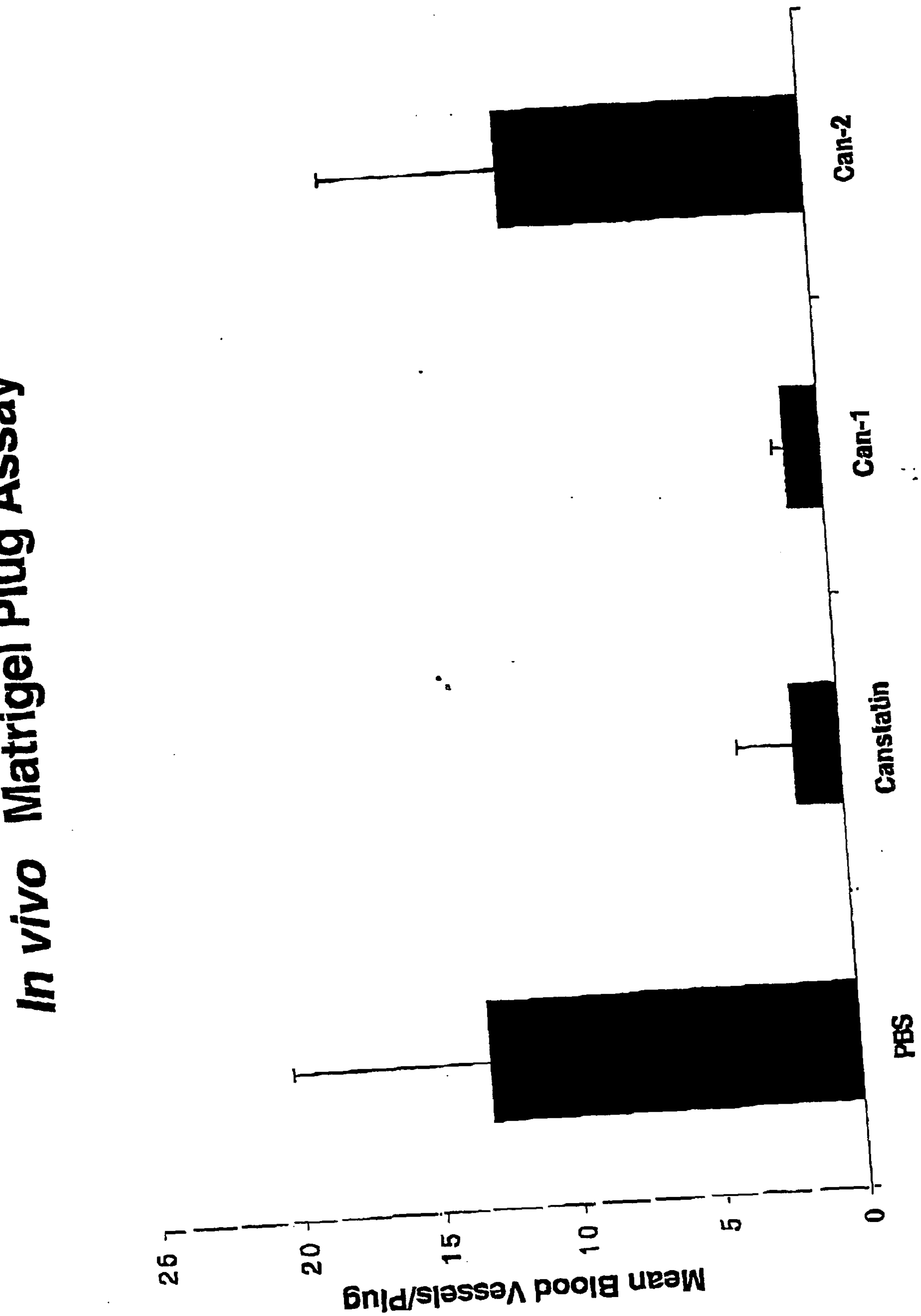


FIG. 41